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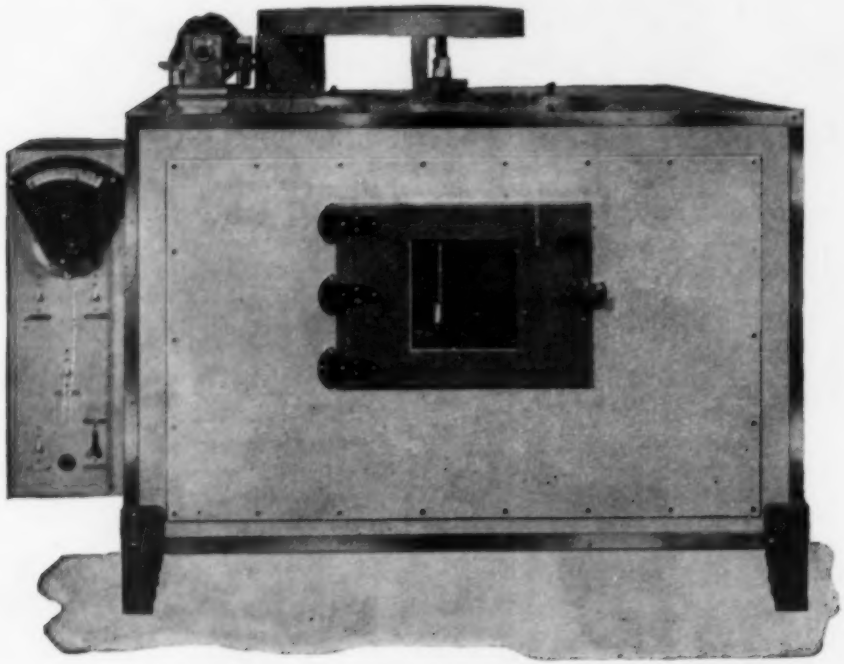

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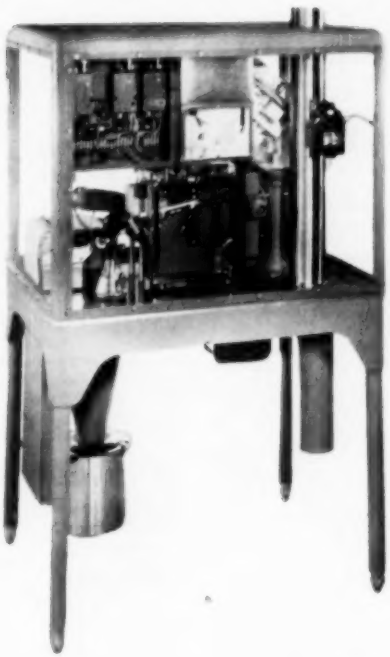

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INSIDE SCIENCE

The Vital Story

A Quick History. Independent investigators, working separately to unlock several of nature's doors, sometimes open up unsuspected relationships. This happened with vitamin B_2.

Investigations. About 25 years ago, several groups, notably Warburg's, were investigating a "yellow enzyme" obtained from yeast. Almost simultaneously other investigators were studying a food factor that aided growth of laboratory animals.

What they found. Proceeding with chemical analysis of this growth factor, the team of Kuhn, György, and Wagner-Jauregg noted a relationship between the growth-producing agent and the "yellow enzyme." Their findings, and those of other researchers along similar lines, were published in 1933. Eventually, riboflavin and an essential part of the yellow enzyme were found to be identical and the unity of an essential nutrient and cellular metabolism was established.

Isolation of pure riboflavin was achieved by Kuhn and his co-workers, and by Ellinger and Koschara, in 1933.

Nomenclature. Known in the United States as riboflavin, this vitamin has also been called lactoflavin, ovoflavin, hepatoflavin, and vitamin G.

SYNTHESIS

By 1935, two eminent chemists, working separately, had synthesized riboflavin, practically in a dead heat. Prof. Paul Karrer of the University of Zurich, a collaborator of the Hoffmann-La Roche Laboratories, produced the first successful synthesis. Five weeks later Richard Kuhn of Germany announced his synthesis of the vitamin. Prof. Karrer subsequently shared the Nobel Prize in Chemistry for his work in vitamins and carotenoids.

The Karrer synthesis forms the basis for chemical processes in widespread use today by Hoffmann-La Roche and other leading manufacturers throughout the world. Riboflavin is also manufactured today by fermentation methods.

CHEMICAL AND PHYSICAL PROPERTIES

Riboflavin is yellow, slightly water-soluble with a greenish fluorescence and a bitter taste. Its empirical formula is $C_{17}H_{20}N_4O_6$. Vitamin B_2 produced by the Roche process is identical in every way with that occurring in nature.

How does vitamin B_2 work? Riboflavin is a vital part of nature's chain of reactions for utilization of carbohydrate energy. It has been found to be a constituent of many enzyme systems and is thus intimately connected with life processes. It is probably required by the metabolic processes of every animal and bird as well as by many fishes, insects and lower forms of life. (In certain animals, however, the requirement may be synthesized by bacteria within the intestine.)

In the cells riboflavin goes to work attached to a phosphate group. This substance, known as riboflavin-5'-phosphate or flavin mononucleotide, may in turn be attached to still another essential substance, adenylic acid, forming flavin adenine dinucleotide. Either nucleotide then is attached to protein, thereby forming an enzyme, and takes its part in oxidation-reduction reactions.

Requirements in Human Nutrition. As we have seen, vitamin B_2 is essential to life. We have no special storage organs in our bodies for this vitamin, although a certain level is maintained in various tissues, with relatively large amounts found in the liver and kidneys.

MEASURING METHODS

In the beginning, riboflavin activity was described in "Bourquin-Sherman units" and requirements were thought to be very small. Subsequent research showed otherwise. Milligrams of weight became the unit and the Food & Drug Administration of the U. S. Dept. of Health, Education & Welfare has established a minimum daily requirement of 2.0 mg. of riboflavin for all persons 12 or more years old. For infants it is 0.5 mg. These requirements are designed to prevent the occurrence of symptoms of riboflavin deficiency disease. The minimum daily requirement for this vitamin for children from 1 to 12 years has not been established by the F. & D. A.

Recommended allowances. The Food & Nutrition Board of the National Research Council has recommended the following daily dietary allowances of riboflavin, expressed as milligrams. These are designed to maintain good nutrition of healthy persons in the U. S. A.

Men		1.6	
Women		1.4	
"	(3rd trimester of pregnancy)	2.0	
"	(Lactating)	2.5	
	Infants, 1-3 months	0.4	
	" 4-9 "	0.7	
	" 10-12 "	0.9	
Children, 1-3 years		1.0	
" 4-6 "		1.2	
" 7-9 "		1.5	
		Boys	Girls
Adolescents, 10-12 years		1.8	1.8
" 13-15 "		2.1	2.0
" 16-20 "		2.5	1.9

# of VITAMIN B_2 *by* Science Writer

(Riboflavin)

Deficiencies of vitamin B_2 appear in several ways in human beings. The eyes, the skin, the nerves, and the blood show the effects of too little riboflavin. Laboratory animals have demonstrated that a riboflavin-deficient diet can cause death of adults and can slow or stop growth in the young. Female animals, deprived of riboflavin in the diet, may produce offspring with congenital malformations.

Medical uses. To overcome and control deficiencies in human beings, physicians have pure riboflavin available for administration by injection or orally, by itself or with other "B" vitamins or multi-vitamin-mineral combinations.

How do we get our daily riboflavin? Vitamin B_2 has wide distribution throughout the entire animal and vegetable kingdoms. Good sources are milk and its products, eggs, meats, legumes, green leaves and buds. Whole-grain cereals have significant but not large amounts of riboflavin.

ADDITION TO FOODS

Cereal foods play a large part in our diet. To produce the white flour almost all of us want, millers are obliged to remove parts of the wheat that contain much of the grain's riboflavin and other nutrients. In addition, cereal grains are not rich sources of riboflavin. Millers meet this problem by *enriching* the grain foods for which federal standards exist with vitamins B_1, B_2, niacin and the mineral iron. In the case of vitamin B_2, however, they do more than *restore* the processed food to its natural riboflavin level; they *fortify* the food with enough of this essential vitamin to make it nutritionally more valuable than it was in nature.

Acting to protect the good health of millions of Americans, bakers and millers adopted *enrichment* of white bread and white flour in 1941. Since that time, other foods, such as macaroni products, corn meal and grits, farina, pastina and breakfast cereals have had their food value increased by enrichment with pure riboflavin and other vitamins and minerals.

When enriching, fortifying or restoring, food manufacturers add the necessary quantity of riboflavin (and other vitamins and minerals) to the food during processing, so that the finished product meets federal, state, and territorial requirements or contributes to the consumer an amount of the vitamin that dietary experts believe significantly useful.

PRODUCTION

Prof. Karrer's synthesis of riboflavin was a laboratory success. Adapting the process to commercial production, however, demanded original thinking by chemists at Hoffmann-La Roche. The production of riboflavin by chemical synthesis requires the production of ribose, a rare sugar, at an early stage in the process. This special sugar must be made inexpensively if the synthesis is to be practical. Sugar chemistry is a difficult matter. In a brilliant piece of work, the Roche chemical experts developed a method to produce ribose on a commercial scale by an electrolytic process, thus overcoming a most troublesome problem. Subsequently, Roche chemists developed the first practical synthesis for riboflavin-5'-phosphate, identical with natural flavin mononucleotide.

Picture three streams joining to form a river and you have a simplified idea of the Roche process for synthesizing vitamin B_2. O-xylene and glucose are processed separately to form xylidine and ribose respectively. These are joined to form ribitylxylidine, which is then converted to ribitylaminoxylidine. Starting separately with malonic ester, which is processed through intermediate stages to alloxan, the third "stream" is then joined with ribitylaminoxylidine to form riboflavin. Purification occurs at each step of the synthesis. Riboflavin 'Roche' equals or exceeds U. S. P. standards.

By the tons. So efficient is the Roche process that pure riboflavin is produced *by the tons* for use in pharmaceutical products and processed foods. An interesting development by Roche is the production of riboflavin in different forms related to the method of end use. 'Roche' Regular riboflavin U. S. P. is especially useful in dry enrichment premixes, powdered dietary supplements, pharmaceutical tablets and soft gelatin capsules. 'Roche' Solutions type is preferred for the manufacture of solutions having low concentration. 'Roche' Riboflavin-5'-Phosphate Sodium is a highly and rapidly soluble riboflavin compound favored for all pharmaceutical liquid products and some tablets, lozenges, and capsules. It has a more pleasant taste than the bitter U. S. P. riboflavin.

This article is published in the interests of pharmaceutical manufacturers, and of food processors who make their good foods better using pure riboflavin 'Roche.' Reprints of this and others in the series will be supplied on request without charge. Also available without cost is a brochure describing the enrichment or fortification of cereal grain products with essential vitamins and minerals. These articles and the brochure have been found most helpful as sources of accurate information in brief form. Teachers especially find them useful in education. Regardless of your occupation, feel free to write for them. Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul St., West, Montreal, Que.

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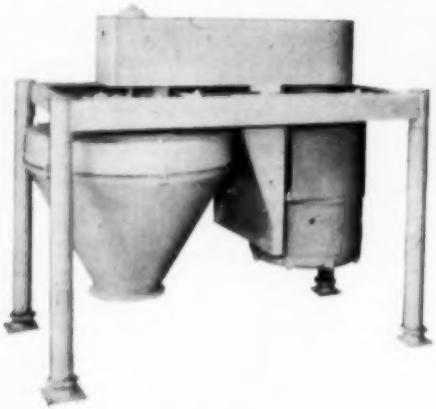

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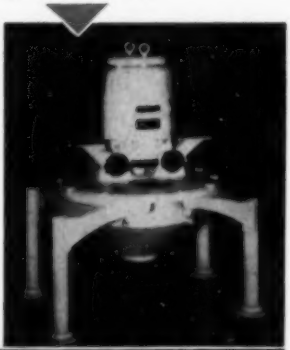

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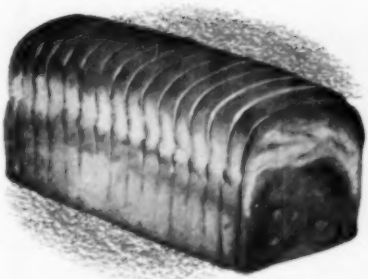

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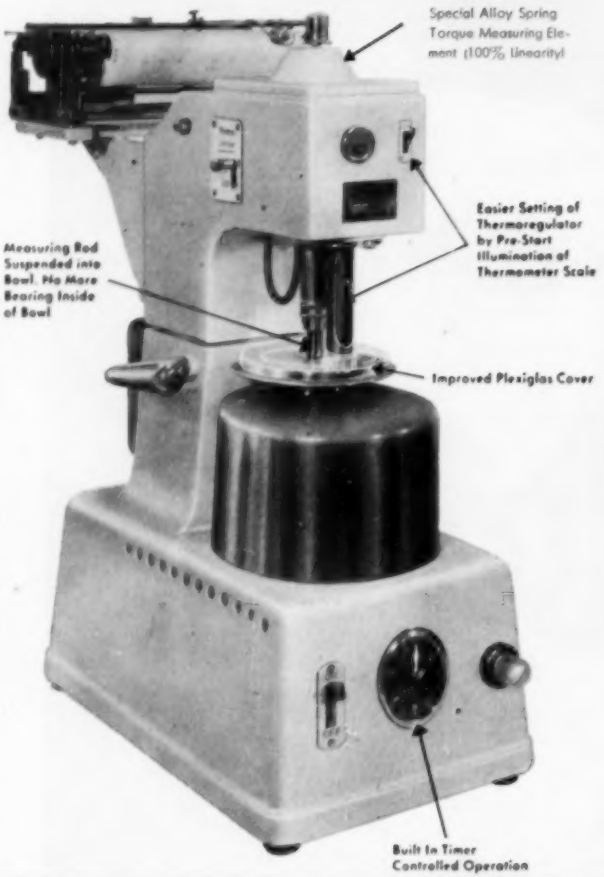
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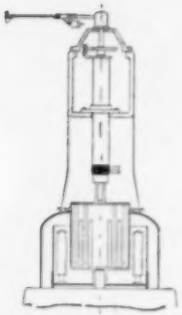

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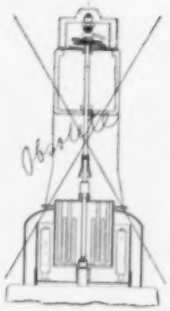

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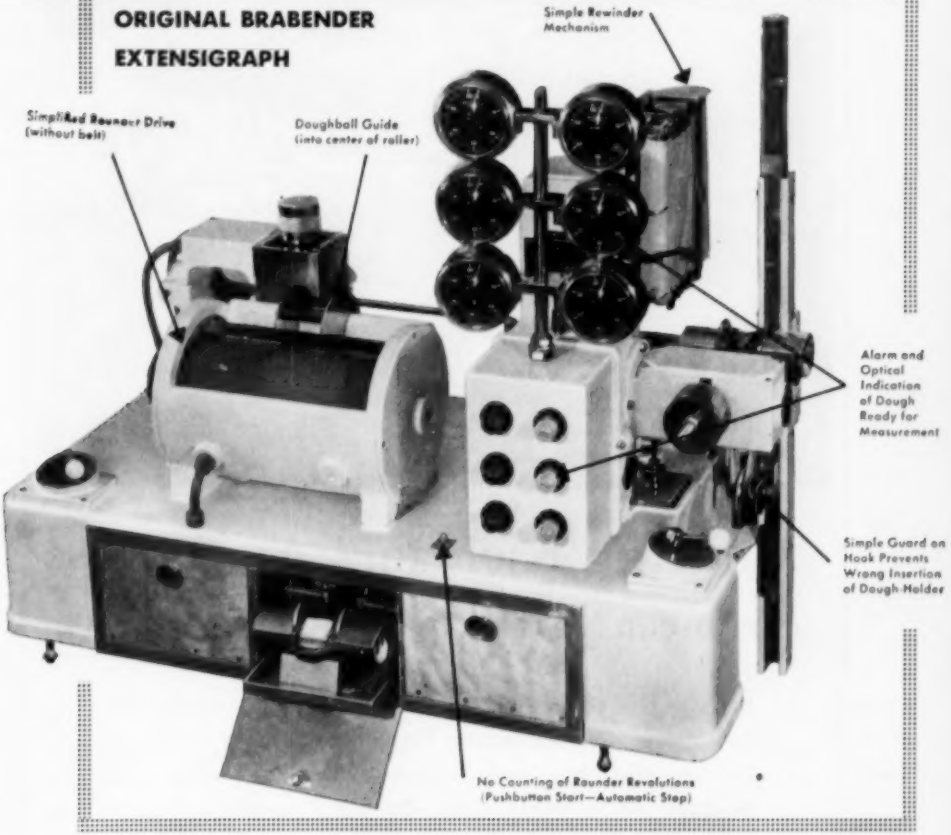
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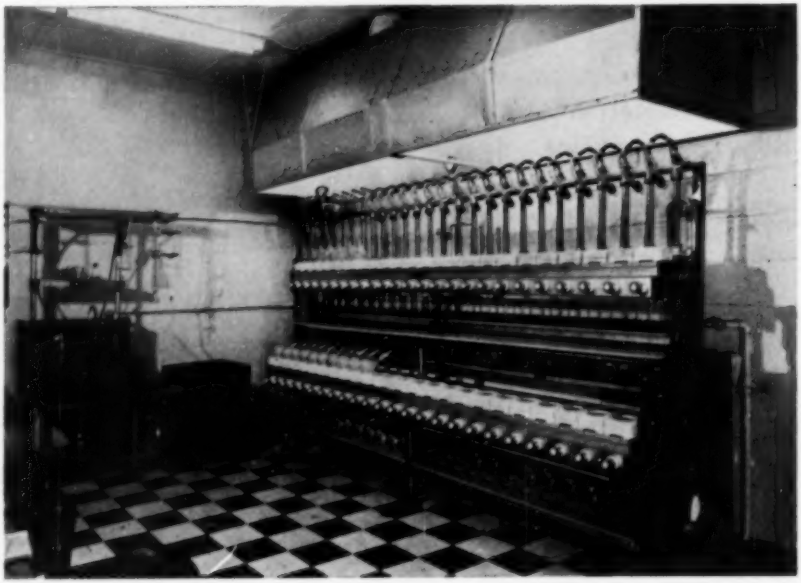

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CEREAL CHEMISTRY

VOL. 34 JULY, 1957 NO. 4

ENZYMES THAT DEGRADE BARLEY GUMS. V. RELATION BETWEEN ENDO-BETA-POLYGLUCOSIDASE ACTIVITY AND OTHER BARLEY AND MALT PROPERTIES[1]

E. J. BASS, V. M. BENDELOW,[2] AND W. O. S. MEREDITH

ABSTRACT

Studies of fifteen barley varieties grown at five stations in 1956 showed that cytolytic activity of green malt, as measured by endo-beta-polyglucosidase activity, is a varietal characteristic. In general, high cytolytic activity was associated with high values of other malt properties. Intervarietal correlation coefficients between all pairs of the following properties were highly significant: cytolytic activity, saccharifying activity, alpha-amylase activity, index of protein modification, cold-water extract, and malt extract. As the highest correlations were between cold-water extract and the other properties, it is suggested that cold-water extract provides one of the best single measures of general malting quality presently available. Evidence is presented to support the hypothesis that cytolysis contributes indirectly to the production of extract by exposing starch and protein to the action of amylases and proteolytic enzymes. The present viscometric determination of cytolytic activity may be used to assist in evaluating malting quality, but additional study is necessary to clarify two conflicting features of this determination.

A previous investigation (3) has shown that the cytolytic activity of green malt, as determined viscometrically with a pure barley beta-polyglucoside as substrate, is a varietal characteristic related to malting quality. In the present investigation a larger number of samples was studied, and additional tests were undertaken to compare endo-beta-polyglucosidase activity with other barley and malt properties. As it has been suggested earlier (2) that endo-beta-polyglucosidase may well be the chief cytolytic enzyme, endo-beta-polyglucosidase activity will be referred to as "cytolytic activity."

Materials and Methods

Fifteen barley varieties were selected for this study. These were: Montcalm, O.A.C. 21, and Parkland, representing good malting quality;

[1] Manuscript received November 21, 1956. Paper No. 160 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg; Paper No. 340 of the Associate Committee on Grain Research; and paper No. 215 of the Cereal Crops Division, Experimental Farms Service, Canada Department of Agriculture.

[2] Chemist, Barley and Malt Laboratory, Department of Agriculture, Winnipeg.

U.M. 570,[3] U.M. 584, Br. 3831, and Br. 3902, representing promising malting quality; Kindred, Traill, and U.M. 913, representing varieties of moderate quality but inferior to O.A.C. 21 by Canadian standards; U.M. 256, Leth. 4323, Br. 3139, Br. 4417, and Vantmore, representing poor malting quality. Samples of the fifteen varieties were obtained from the experimental stations at Winnipeg (Manitoba), Tisdale and Saskatoon (Saskatchewan), and Lacombe and Beaverlodge (Alberta). Barley samples were divided into two portions, one of which was processed in the usual way by the Barley Improvement Institute. The other was malted in 10-g. lots and used for determining cytolytic activity.

The 10-g. samples of barley were malted, in lots of six varieties, in perforated plastic containers of approximately 1-pt. capacity. These were steeped at 10°C. to a standard moisture content (44%), as determined by a pilot steep. They were then germinated at 12°C. for 6 days, after which they were analyzed immediately.

In the present investigation, errors in cytolytic activity arising from differences in initial substrate viscosity were eliminated by adjusting substrate solutions to the same initial viscosity. As before (3), the substrate was a beta-polyglucoside isolated from Montcalm barley. Substrate solutions were prepared by mixing 2 g. of gum with 175 ml. of 0.1 M sodium chloride for 30 minutes in a 90°C. water bath. The mixture was filtered by suction while hot (Whatman 41 H filter paper), and the filtrate was cooled under a stream of cold tapwater. The solution was then adjusted to temperature in a 30°C. water bath, and diluted by successive small additions of distilled water until a reading of 85 was obtained on a Brookfield Viscometer. This reading represents a relative viscosity of approximately 88. Six drops of toluene were added to the final solution, which was then stored in a refrigerator (7°C.) until used. Substrate solutions prepared in this way were stable for at least 5 days, and variations in initial viscosity between substrate solutions prepared at different times were negligible.

Cytolytic activity of green malt was determined as before (3), except that 7 ml. (instead of 10 ml.) of green malt extract were diluted to 100 ml. to give the final enzyme solution. To obtain varietal cytolytic activities of the same magnitude as before, cytolytic activity was defined as 120 times the slope of the linear reaction curve obtained by plotting reciprocal specific viscosity vs. time.

The minor modifications introduced into the present malting and analytical procedures improved the precision of the enzymatic determination. Thus, the standard error of a single determination and the

[3] U.M. 570 has recently been classed as not equal to O.A.C. 21 in malting quality because of excessively high wort nitrogen and diastatic activity.

necessary difference between varietal means were reduced by 33% and 13% of their earlier values (3).

Barley cytolytic activity was determined in much the same way. Differences in procedure were that barley was ground in a Wiley mill (1-mm. sieve) before extraction, barley extracts were used without dilution in reaction mixtures, and reaction mixtures contained 3 ml. of enzyme solution instead of 1 ml. enzyme and 2 ml. buffer. Hence to convert barley cytolytic activity to the same basis as green malt cytolytic activity, barley activity was multiplied by the factor 7/300.

Standard barley and malt analyses were made according to A.S.B.C. procedures (1). Cold-water extract was determined by mashing 25 g. malt with 195 ml. water and 5 ml. 0.05 M mercuric chloride at 18°C. for 120 minutes. The mixture was then filtered and the extract was determined from the specific gravity of the filtrate.

Results

Table I shows the cytolytic activities of the green malts, and Table II shows the analysis of variance for these data. The standard error of a single determination was 0.04 units, and the necessary difference between varieties was 0.07 units. As in the previous study, varietal differences in cytolytic activity were highly significant. Thus, with O.A.C. 21 representing the standard, the varieties may be divided into four groups according to activity: high, equal to the standard, moderate, and low. One variety, U.M. 570, had an activity significantly higher than O.A.C. 21; four varieties, Montcalm, U.M. 584, U.M. 913, and Br. 4417, were equal to O.A.C. 21; five varieties, Br. 3902, Parkland, Br. 3831, Kindred, and Traill, had moderate activities just significantly

TABLE I

CYTOLYTIC ACTIVITIES OF THE GREEN MALTS OF THE BARLEY VARIETIES

VARIETY	WINNIPEG	LACOMBE	TISDALE	BEAVERLODGE	SASKATOON	MEAN
U.M. 570	0.68	0.50	0.63	0.53	0.52	0.57
U.M. 584	.55	.51	.72	.40	.49	.53
Montcalm	.57	.48	.60	.41	.52	.52
O.A.C. 21	.49	.50	.55	.38	.41	.48
U.M. 913	.48	.42	.56	.39	.42	.45
Br. 4417	.37	.36	.52	.48	.38	.42
Br. 3902	.37	.33	.48	.43	.46	.41
Parkland	.40	.31	.53	.41	.35	.40
Br. 3831	.42	.31	.52	.40	.33	.40
Kindred	.38	.35	.49	.42	.35	.40
Traill	.45	.26	.48	.32	.44	.39
Br. 3139	.38	.27	.43	.29	.28	.33
U.M. 256	.37	.22	.31	.32	.28	.30
Vantmore	.25	.16	.25	.25	.32	.25
Leth. 4323	.26	.12	.25	.23	.29	.23
Mean	0.43	0.34	0.49	0.38	0.39	0.40

lower than O.A.C. 21; the remaining four varieties, Br. 3139, U.M. 256, Vantmore, and Leth. 4323, were decidedly inferior to O.A.C. 21 in the cytolytic activity of their green malts.

TABLE II

ANALYSIS OF VARIANCE OF CYTOLYTIC ACTIVITY DATA

VARIANCE	DEGREES OF FREEDOM	MEAN SQUARES
Due to varieties	14	0.0487**
Due to stations	4	0.0474**
Due to varieties × stations	56	0.0028

A preliminary inspection of the data in Table I shows that there is a relation between cytolytic activity and general malting quality. Accordingly, cytolytic activity of barley, cytolytic activity of green malt, and general malting quality are compared in Table III. Barley cytolytic activity varies from 0.0020 to 0.0040 units, with thirteen of the fifteen varieties showing activities of 0.0027 ± 0.0005 units. Consequently barley cytolytic activity does not appear to differentiate varieties. Moreover, barley cytolytic activity is not related to general malting quality or to cytolytic activity of green malt. This agrees with the conclusion reached by various European investigators (5, 6, 7, 10) that barley cytolytic activity is not a quality characteristic. However, cytolytic activity of green malt appears to be related to general malting quality, hence a comparison between cytolytic activity of green malt and other malt properties appeared justified.

TABLE III

COMPARISON OF CYTOLYTIC ACTIVITY WITH GENERAL MALTING QUALITY

VARIETY	CYTOLYTIC ACTIVITY		GENERAL MALTING QUALITY
	Barley	Green Malt	
U.M. 570	0.0024	0.57	promising
U.M. 584	.0030	.53	promising
Montcalm	.0020	.52	good
O.A.C. 21	.0028	.48	good
U.M. 913	.0023	.45	inferior
Br. 4417	.0040	.42	inferior
Br. 3902	.0028	.41	promising
Parkland	.0025	.40	good
Br. 3831	.0022	.40	promising
Kindred	.0023	.40	inferior
Traill	.0026	.39	inferior
Br. 3139	.0033	.33	poor
U.M. 256	.0032	.30	poor
Vantmore	.0032	.25	poor
Leth. 4323	0.0028	0.23	poor

Table IV shows a comparison between the cytolytic activity of green malt and the following kilned malt properties: saccharifying activity,

alpha-amylase activity, cold-water extract, extract, and index of protein modification. In general, high cytolytic activities were associated with high values of the other malt properties, suggesting that all the properties in Table IV were interrelated. This was confirmed by calculating the correlation coefficients shown in Table V. Of the fifteen coefficients relating all possible pairs of the six properties, fourteen were significant to the 1% level, and one coefficient (extract × alpha-amylase) was significant to the 5% level.

TABLE IV

COMPARISON OF CYTOLYTIC ACTIVITY OF GREEN MALT WITH MALT PROPERTIES[a]

Variety	Green Malt	Kilned Malt				
	Cytolytic Activity	Saccharifying Activity	Alpha-amylase Activity	Cold-Water Extract	Malt Extract	Index of Protein Modification [b]
		°L.	20°D.U.	%	%	
U.M. 570	0.57	229	58.0	20.8	74.1	40.7
U.M. 584	.53	183	48.8	19.1	74.4	36.6
Montcalm	.52	187	44.9	19.1	74.6	37.5
O.A.C. 21	.48	182	45.5	19.0	73.4	36.7
U.M. 913	.45	183	31.2	18.7	72.9	36.4
Br. 4417	.42	168	47.5	17.8	68.4	34.6
Br. 3902	.41	168	44.7	19.2	75.1	40.3
Parkland	.40	172	43.6	19.4	75.2	39.7
Br. 3831	.40	175	44.7	19.5	74.9	38.2
Kindred	.40	197	44.2	18.9	72.4	38.7
Traill	.39	179	39.6	17.3	72.9	35.4
Br. 3139	.33	129	40.0	16.5	70.4	34.4
U.M. 256	.30	161	25.0	16.9	71.6	31.6
Vantmore	.25	111	21.3	14.3	66.6	28.1
Leth. 4323	0.23	81	19.2	13.6	68.4	30.2

[a] Mean value over all stations.

[b] Index of Protein Modification $= \frac{\%\ \text{wort N} \times \%\ \text{extract}}{\%\ \text{barley N}}$.

TABLE V

INTERVARIETAL CORRELATION COEFFICIENTS FOR MALT PROPERTIES

	Cytolytic Activity	Saccharifying Activity	Alpha-Amylase Activity	Cold-Water Extract	Index of Protein Modification
Extract	0.71**	0.72**	0.64*	0.85**	0.86**
Index of Protein Modification	0.79**	0.80**	0.85**	0.93**	...
Cold-water extract	0.88**	0.93**	0.86**	...	...
Alpha-amylase activity	0.85**	0.80**	...	...	...
Saccharifying activity	0.87**	...	...	...	...

General Discussion

Two interesting results emerge from the present investigation. First, the cold-water extract apparently provides one of the best single tests for general malting quality currently available. Second, cytolytic ac-

tivity of green malt is a fundamental varietal characteristic indirectly involved in the production of malt extract. These conclusions merit further discussion from both practical and theoretical viewpoints.

The first conclusion is substantiated by a consideration of the correlation coefficients in Table V. According to these data, the closest relations are between cold-water extract of kilned malt and the following: index of protein modification (0.93**), saccharifying activity (0.93**), cytolytic activity of green malt (0.88**), and alpha-amylase activity (0.86**). Thus cold-water extract reflects both the extent of proteolysis and the development of carbohydrase activity, during the malting process. As these changes, among others, are involved in malt modification, cold-water extract appears to be a practical measure of the extent of modification occurring during the malting process. Moreover, because there is a high correlation between cold-water extract and malt extract (0.85**), cold-water extract also reflects the amounts of materials made soluble by congress mashing. Hence cold-water extract is an excellent test for general malting quality.

The second conclusion arises from a consideration of the possible significance of selected correlation coefficients in Table V. The high correlations between extract or cold-water extract and saccharifying activity, alpha-amylase activity and index of protein modification are not surprising. Amylolytic and proteolytic enzymes are directly involved in the production of "congress" extract, whereas proteolytic enzymes are directly involved in the production of cold-water extract. However, similarly high correlations between cytolytic activity and extract or cold-water extract cannot be explained on the same basis, as the direct contribution of cytolysis to extract is estimated, on the basis of malt gum content, to be less than 5%. Consequently cytolysis is indirectly involved in the production of extract.

A view that has long been held by various investigators (8, 9) is that cytolytic enzymes rupture endosperm cell walls during germination, thus exposing the cell contents to proteolytic and amylolytic action. This hypothesis satisfies the above requirements of a process that is closely related to, but not directly involved in, extract production. To date, the only convincing evidence favoring this view of the process of modification is the histological investigation of Dickson and Shands (4), which showed that during germination the dissolution of endosperm cell walls precedes dissolution of the proteinaceous matrix embedding the starch granules. Thus the present investigation provides additional evidence for the hypothesis that cytolysis is a necessary prerequisite to amylolysis and possibly also to proteolysis.

Although the present viscometric determination of cytolytic activity

provides fundamental information that can assist in evaluating malting quality, this determination has revealed two features requiring further study. First, in the varieties Parkland, Br. 3902, and Br. 3831, relatively low cytolytic activity is associated with satisfactory malting properties. On the other hand, in the varieties U.M. 913 and Br. 4417 relatively high activity is associated with one or more unsatisfactory malting properties. Studies of factors of secondary importance in cytolysis are now in progress, and there is reason to believe that these studies may resolve the first type of anomaly, and lead to a more useful determination of cytolytic activity than is provided by endo-beta-polyglucosidase activity alone.

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APPLICATION OF THE FAT ACIDITY TEST AS AN INDEX OF GRAIN DETERIORATION[1]

DORIS BAKER, M. H. NEUSTADT, AND LAWRENCE ZELENY[2]

ABSTRACT

A rapid and simple method for determining fat acidity in grain has been developed, utilizing a grinder-extractor to shorten the extraction time. On comparison with the official A.O.A.C. method, the rapid method gave slightly lower values (0.3 units for wheat and 0.5 units for barley and rye). Fat acidity surveys of several hundred samples of sound and damaged grain have led to the establishment of maximal fat acidity values for grain showing little or no deterioration. These limits are 10 for beans and peas, 22 for soybeans, and 20 to 25 for cereal grains except oats. Damaged grain showed higher values depending upon the degree and type of damage. A curvilinear relationship was found to exist between fat acidity and the moisture content of the grain at the time of extraction. Wheat and barley in the range of 10 to 17% moisture exhibit a 1.0 to 1.5 unit increase in fat acidity for each percent increase in moisture. To secure comparable results all samples should be analyzed at a moisture content not exceeding 10%, or appropriate correction factors may be applied to the fat acidity values.

The fat acidity test was developed (5, 6) as an objective index of the degree of soundness in grain. Fat acidity is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids from 100 g. of grain, calculated on a moisture-free basis. The test is an official method of analysis of the Association of Official Agricultural Chemists (1). A more rapid and simpler method utilizing a grinder-extractor has been developed.

Method

Reagents. (a) Benzene, purified grade.

(b) Alcohol-phenolphthalein solution in 95% ethanol.

(c) Potassium hydroxide, 0.0356*N* (1 ml. = 2 mg. potassium hydroxide).

(d) Potassium dichromate, 0.5% solution.

(e) Potassium permanganate, 0.01% solution.

Apparatus. (a) Grinder-extractor, Stein Mill[3] Model M1 or equivalent (Fig. 1).

(b) Buret or bottle with graduated dropper (1.5 to 2.5 ml. capacity).

Procedure. Determine the moisture content of the sample to be analyzed.

[1] Manuscript received May 29, 1956. The study upon which these findings are based was made under authority of the Research and Marketing Act of 1946.

[2] Grain Division, Agricultural Marketing Service, United States Department of Agriculture, Washington 25, D.C.

[3] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

Weigh 40.0 g. of a representative sample of whole grain into the cup of the grinder-extractor. Attach cup to mill and run the mill 1

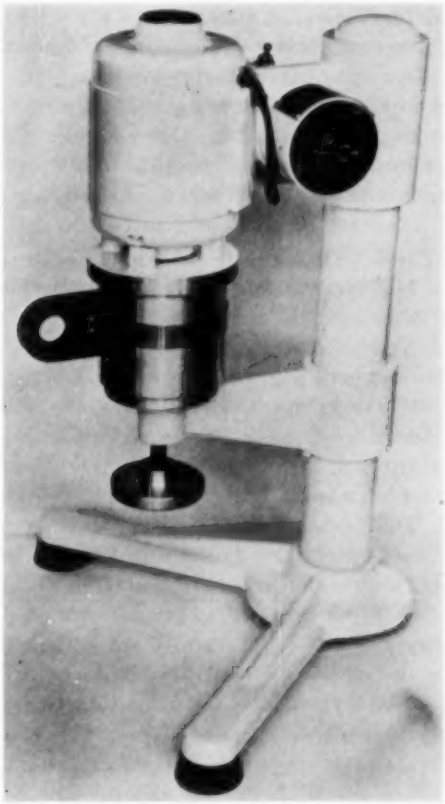

Fig. 1. Stein Mill, Model M1.

minute. Carefully lower cup and add 100 ml. benzene. Replace cup and run mill 4 minutes more. Remove cup from mill and immediately decant liquid into filter. Cover filtering funnel with watch-glass while filtering. Using an accurately graduated cylinder, measure a 25-ml. aliquot of the filtrate and transfer to titration flask (125-ml. Erlenmeyer). Again fill the graduate with 25 ml. of the alcohol-phenolphthalein solution and add to the 25 ml. in the titration flask. Titrate the solution with 0.0356*N* potassium hydroxide, using either a buret or graduated dropper. The end point should match the color of a solution made by adding 2.5 ml. of 0.01% potassium permanganate

solution to 50 ml. of potassium dichromate solution of proper strength to match the color of the original solution being titrated. (Add 0.5% potassium dichromate solution dropwise to 50 ml. of water until color of the extract-alcohol solution is matched. Then add 2.5 ml. of the 0.01% potassium permanganate solution. This final solution may be used as standard for titration endpoint.) Make a blank titration on 50 ml. of the 1:1 benzene-alcohol-phenolphthalein solution and subtract this value from the titration value of the sample. Calculate the fat acidity as the number of mg. of potassium hydroxide required to neutralize the free fatty acids from 100 g. of dry grain by the formula:

$$\text{Fat acidity} = \frac{(\text{titration-blank}) \times 2 \times 10}{100 - \%\ \text{moisture in sample}} \times 100$$

Discussion of the Method. Using the rapid method, one operator can run a single determination within 10 minutes after a representative sample of grain has been obtained. Six to eight determinations can be made in an hour depending upon the operator.

The rapid method was compared with the official method by making fat acidity determinations on 37 samples of wheat, 20 samples of barley, and 20 samples of rye. The wheat samples ranged in fat acidity from 8 to 52, the barley from 6 to 32, and the rye from 9 to 40 by the official method. Duplicate determinations by both methods agreed within 2 fat acidity units. The range in difference between the two methods was from 0 to 4 fat acidity units. The mean difference between the two methods was 0.3 unit for wheat and 0.5 unit for barley and rye. Usually very slightly lower results are obtained by the rapid method than by the official method. The standard deviation between the two methods was 2 fat acidity units for these three grains.

In order to maintain the closest agreement between duplicates and between the two methods it is quite important to obtain a representative sample of grain for analysis. The sample should be well mixed, and it is desirable to obtain the portion for analysis with the aid of a mechanical divider. For the small grains it is usually possible to obtain uniform results by using a 40-g. portion for analysis. With larger grains such as corn it is more difficult to obtain a representative sample of only 40 g. For most accurate results it is recommended that 200 g. of corn be ground first and then the 40 g. taken from the well-mixed ground sample.

Effect of Moisture Content on Fat Acidity Determinations

The influence of moisture content on fat acidity determinations has been noted by the fact that fat acidity values increase or decrease with corresponding changes in moisture content at the time of extrac-

tion. If the fat acidity value of a sample of grain is determined at 10% moisture and then again after the grain has been tempered to 14% moisture, it will show a higher fat acidity value at the higher moisture level. Similarly, if the fat acidity value is determined at 14% moisture and then the grain is dried to 10% moisture, the fat acidity value will be lower at the 10% moisture level. This relationship was first observed in pea beans which, when kept in ventilated storage, decreased in fat acidity value as the moisture content decreased. It has since come to our attention that others working in this field have made similar observations. This led to an investigation of other grains where a similar relationship was observed. To obtain sufficient quantities of oil to test, 400-g. portions of wheat and corn were extracted in a large Soxhlet extractor using Skellysolve F. The grain was first extracted at a low moisture content and then additionally extracted after water had been added to raise the moisture content to at least 20%. Not only is the oil from the wet extraction different in appearance, but a difference in composition is evident from the analysis given in Table I. The higher percentages of nitrogen and phosphorus in the oil from the wet extraction indicates more phospholipide present than in the oil

TABLE I

ANALYSIS OF OIL FROM DRY AND WET EXTRACTIONS

SAMPLE	MOISTURE	WEIGHT OF OIL EXTRACTED	IODINE NO.	ACID VALUE	NITROGEN	PHOSPHORUS
	%	g.			%	%
Wheat:						
Dry extraction	6.0	5.33	126.9	8.2	0.2	0.27
Additional wet extraction	20-22	1.20	104.7	32.5	1.0	1.46
Corn:						
Dry extraction	7.9	17.79	118.9	3.1	...	Absent
Additional wet extraction	20-22	0.73	90.3	75.7	...	Present

from the dry extraction. According to Eckey (2) the phospholipide is believed to be combined with the protein in such a way that it is not extracted by most fat solvents, but alcohol or water will loosen the combination. The phospholipides could then have broken down to give fatty acids and glycerophosphoric acid to account for the higher acid value of the oil from the wet extraction. The lower iodine value of the oil from the wet extraction indicates that the acidity is not all caused by extraction of more fatty acids alone. Therefore the presence of phosphoric acid in some form is indicated.

If the fat acidity value is to be used as an index in grain deterioration, it is important only as it applies to those fatty acids originally present or present as the result of normal deterioration. Any fatty

acids or other titratable material introduced by hydrolysis at the time of extraction would not indicate the true fat acidity value of the grain.

In order to study the relationship between grain moisture content and fat acidity, several series of determinations were run on samples of grain at different moisture levels. Samples of grain between 500 and 1600 g. were tempered to higher moisture levels by adding calculated amounts of water, mixing, and letting stand in a refrigerator at least 72 hours. The samples were then dried at temperatures not exceeding 110° C. and portions removed periodically as the grain dried. In this manner series of subsamples covering a wide range in moisture content were obtained. Fat acidities were determined by the official method, starting the extraction on the same day the samples were dried. The moisture contents were determined by the 130° C. air-oven method on the ground samples used for the fat acidity determinations. Examples of some of these series of determinations are given in Table II.

TABLE II
FAT ACIDITY VALUES AT DIFFERENT MOISTURE LEVELS

Wheat		Barley		Corn		Soybeans	
Moisture	Fat Acidity	Moisture	Fat Acidity	Moisture	Fat Acidity	Moisture	Fat Acidity
%		%		%		%	
17.4	25.4	17.1	21.1	22.9	40.6	16.3	38.8
15.2	23.0	14.9	18.8	20.7	35.0	14.5	38.6
13.8	19.7	13.0	14.4	17.2	32.9	13.4	33.3
12.5	16.6	11.5	13.6	16.0	32.5	13.1	34.5
11.0	15.7	10.9	12.3	15.0	25.3	11.9	32.7
10.9	16.8	9.6	13.0	13.7	22.9	10.9	31.4
10.2	15.6	8.8	11.5	12.1	19.9	9.5	30.1
9.3	14.9	8.0	12.3	12.0	19.3	8.8	31.6
7.9	14.4	7.2	12.4	11.1	17.4	7.7	27.6
6.8	14.5	6.8	13.4	10.3	17.1	6.5	28.4

When these results are graphed a curvilinear relation between moisture content and fat acidity value is obtained. From a series of ten of these curves, average correction factors were determined for wheat and barley. At 10 to 12% moisture the average rate of change is 1.0 fat acidity units per percent change in moisture content. At 12 to 17% moisture the average rate of change is 1.5 fat acidity units. Below 10% moisture the average rate of change is 0.5 fat acidity unit per percent change in moisture content, which is small enough to be considered insignificant for most practical purposes, especially since very little grain in storage is below 9% moisture.

In the case of corn and soybeans the samples did not show enough consistency to establish a reliable correction factor above 10% moisture. Therefore both corn and soybeans should be dried to 10% moisture

before the fat acidity value is determined. It is hoped that further investigation will shed more light on this subject.

Fat Acidity Surveys

To study the fat acidity levels characteristic of freshly harvested sound grain and other related commodities, a total of 1205 samples of wheat, corn, barley, oats, rye, grain sorghums, flaxseed, soybeans, rough rice, dried beans, and dried peas representing the 1951, 1952, and 1953 crops were obtained from agricultural experiment stations in 26 states and the General Field Headquarters office of the Grain Division. Fat acidity tests were made by the official method immediately after the samples were received. The moisture content of these samples was generally low, so that there was no necessity to apply a correction factor.

Based on the data obtained (4), fat acidity values have been established below which nearly all freshly harvested grain of unquestionable soundness is believed to fall. These fat acidity limits and the percentages of the 1951-, 1952-, and 1953-crop samples surveyed falling below these limits are shown in Table III. It is believed that in the 1951 survey some of the samples received did not represent freshly harvested grain. Soybeans, flaxseed, dried beans, and dried peas are not generally classed as grain but were included in this survey because they are covered by standards administered by the Grain Division.

TABLE III

FAT ACIDITY SURVEY OF SOUND GRAIN

KIND OF GRAIN	TOTAL NUMBER OF SAMPLES	AVERAGE FAT ACIDITY VALUE (THREE CROP YEARS)	FAT ACIDITY LIMIT[a]	PERCENTAGE OF SAMPLES THAT FELL BELOW FAT ACIDITY LIMIT			
				1951	1952	1953	Total
Wheat	225	10.9	20	98	99	100	99.6
Corn	84	14.9	22	96	100	98	97.6
Barley	161	13.3	25	93	100	100	97.5
Oats	158	42.3	60[b]	85	98	94	92.4
Rye	74	15.6	22	100	100	95	98.6
Grain sorghums	104	14.0	25	93	100	100	95.2
Flaxseed	88	42.7	40[b]	42	80	97	77.2
Soybeans	129	11.0	22	97	96	100	97.7
Rice, rough	48	13.4	25[b]	75	89	100	93.8
Beans	109	6.0	10	98	100	98	98.2
Peas	25	4.9[c]	10[b]		100	100	100.0

[a] Fat acidity limit below which nearly all freshly harvested grain of unquestionable soundness is believed to fall.
[b] These limits should be considered as very tentative.
[c] Two crop years only.

Studies have also been made on grains representing different degrees and types of damage. Although these data are not entirely complete, enough samples have been collected representing certain types

of damage to show the relationship between the fat acidity value and degree of damage. This relationship has also been observed by others working in this field (3). A good correlation has been obtained between percent damage and fat acidity value for "sick" wheat, and for corn when the damage is caused by blue-eye mold, cob rot, or heat. Coefficients of correlation for some types of damage are shown in Table IV.

TABLE IV

CORRELATION COEFFICIENTS BETWEEN FAT ACIDITY AND SOME TYPES OF DAMAGE

GRAIN	TYPE OF DAMAGE	NUMBER OF SAMPLES	DAMAGE			FAT ACIDITY UNITS			CORRELATION
			Min.	Max.	Mean	Min.	Max.	Mean	
			%	%	%				
Wheat	"Sick" wheat	38	2.2	100	23.7	11	66	31	+0.81**
	Blight, mold, scab	14	2.4	100	24.7	12	84	23	+0.70**
	Frost	13	3.2	100	23.3	9	18	13	+0.63*
Corn	Heat	33	1.0	100	40.8	21	275	84	+0.66**
	Blue-eye mold	93	0.8	100	16.8	21	284	63	+0.85**
	Cob rot	13	3.0	100	35.9	19	272	113	+0.99**

The extent to which fat acidity values rise is evident in these studies. Maximum fat acidity values found for various types of damage are shown in Table V.

TABLE V

MAXIMUM FAT ACIDITY VALUES

GRAIN	TYPE OF DAMAGE	MAXIMUM FAT ACIDITY VALUE
Wheat	"Sick" wheat	66
	Blight, mold, and/or scab	84
Corn	Heat	275
	Blue-eye mold	284
	Cob rot	272

General Discussion

The fat acidity test, if properly handled and interpreted, shows evidence of being a good index of grain deterioration. By the rapid method the fat acidity value of a sample of relatively dry grain can be determined within 10 minutes as compared with 16 to 18 hours by the Soxhlet extraction method. Since the fat acidity value is appreciably influenced by a high moisture content, it is recommended that grain samples be dried to 10 percent moisture or less before the fat acidity determination is made, or that the appropriate correction factor be applied where it has been established. It has been shown by the fat acidity surveys that grain of unquestionable soundness will fall below a certain fat acidity limit and that there is a relationship between fat

acidity values and the degree and type of damage. Some types of damage are reflected in the fat acidity test more clearly than others, and further investigation is needed along this line before any definite conclusions can be drawn.

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MEASURING STRESS RELAXATION IN STARCH GELS[1]

C. O. Chichester and Clarence Sterling

ABSTRACT

An apparatus is described for recording continuously the relaxation of stress in starch gels under compression. The apparatus permits the selection of varying amounts of deformation to be imposed on the sample. Stress is developed in strain gages, which are mounted on a steel cantilever beam. A potential, modified in the deformed strain gages, is applied to a millivolt recorder. The linear relationship between beam deformation and gage output permits a direct chronological record of relaxation of stress in the gel.

Among the various approaches which have been found useful in rheological studies of high polymers, the method of stress relaxation has been accorded considerable favor (1, 2, 4, 7). Stress relaxation determinations have been used to analyze molecular organization (7, 15) and to predict the response of materials under conditions of actual practice (11, 15). In a previous paper (6) the authors described the construction of an apparatus for measuring creep and recovery in gels under compression. The present study details a method for recording the relaxation of stress in gels (or other soft materials) held at constant deformation.

Mechanical Apparatus

In the technique used here (see Fig. 1), a steel cantilever beam

[1] Manuscript received July 17, 1956. Contribution from the Department of Food Technology, University of California, Davis, California.

B is mounted firmly on the vertically adjustable sample stage *SS* of a penetrometer. The specimen *S* is placed on a flat platform P_2 at the tip of the beam, and the whole assembly is raised until the upper surface of the specimen is just in contact with the face of a vertically adjustable piston P_1. The piston has been set previously, with a stop *ST* mounted on its shaft, to the nearest 0.1 mm. of deformation *D* desired. (The deformation is set conveniently with the penetrometer dial.) When the clamp spring *CL* is bent by the release lever *R*, the piston can be thrust down quickly to the stop, thus imposing the desired deformation on the gel.

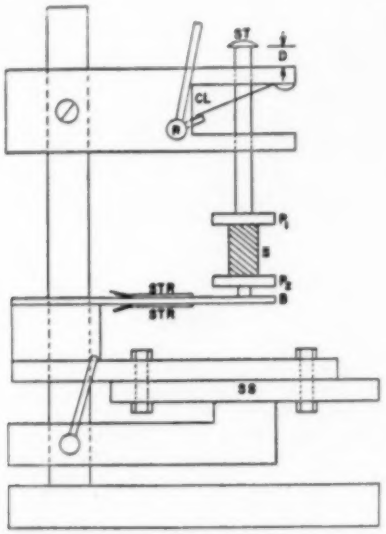

Fig. 1. Diagram of apparatus for imposing strain on the gel. S, gel sample; P_1, piston; P_2, platform with hard, flat-surfaced disks; B, cantilever steel beam; STR, strain gage; SS, adjustable stage; ST, stop; D, amount of deformation to be imposed; CL, clamp spring, released by the lever at R.

Strain gages *STR* which are cemented on the cantilever beam, are deformed as the beam is bent by the stress from the compressed gel. Deformation of a strain gage results in a proportional increase of its impedance to the passage of an electric current. In order to minimize the amount of beam deflection caused by the stress from the gel, the beam is made of a high-carbon tool steel, ground to the final dimensions: 1.295 cm. breadth × 0.335 cm. height × 10.8 cm. length.

The deflection of a rectangular cantilever beam is given by the relationship:

$$d = 4m\ g\ l^3/E\ a^3b,$$

where d is the amount of deflection,
m is the mass of the applied load,
g is the acceleration due to gravity,
l is the length of the beam,
E is Young's modulus (20×10^{11} dynes/cm^2 here),
a is the height of the beam, and
b is the breadth of the beam.

Under the conditions given here, there is a beam deflection of 0.05 mm. per 100 g. of applied load. When 18 volts of direct current are supplied through the strain gages, the full scale of the millivolt recorder (discussed below) corresponds to a maximum load of 318 g. of force, or a beam deflection of 0.16 mm. With tender gels, in which a rather large deformation (of the order of many millimeters) must be imposed in order to attain full-scale values, corrections for beam deflections of this magnitude may be conveniently neglected. It should be noted that there is a rectilinear relation between change in strain and change in output of voltage from the circuit of which the gages are a part (3).

Electrical System

Figure 2 shows the design of the electrical system. Two matched strain gages *STR*, of 490 ohms resistance, are cemented to the top and bottom surfaces, respectively, of the cantilever beam. Each strain gage functions as an arm of a Wheatstone bridge. When the beam is

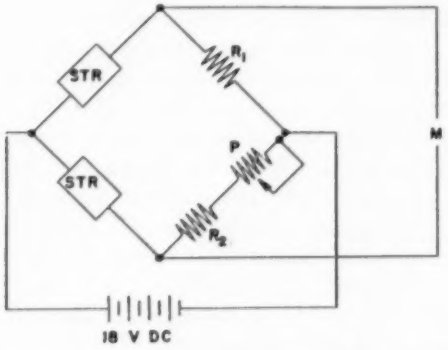

Fig. 2. Diagram of electrical system. STR, strain gage (two in parallel); R_1 and R_2, 493-ohm resistances; P, 5-ohm potentiometer; 18-volt dry cell; M, millivolt recorder.

strained, the upper gage is under traction and the lower under compression, thus giving twice the output potential of a single gage (3).

The remaining arms of the bridge are two 493-ohm, wire-wound resistors (R_1, R_2) which are taped together in a small case to ensure thermal equivalency. A potentiometer of 5 ohms is connected in series with one of the resistors to permit zero-adjustment of the bridge.

The sensitivity of the system varies with the voltage applied to the bridge; in this case an 18-volt potential *(18 V DC)* was used to obtain the necessary sensitivity. The recording potentiometer *M* has a sensitivity of 1 millivolt for full-scale deflection. However, the speed of response of the recording system is governed largely by the speed of the recorder pen. In the instrument used, this was 2.5 seconds for a traversal of the full scale. When the recorder is not in balance with the potential of the bridge, it acts as a shunting resistor across the entire bridge and decreases the speed of the response of the system somewhat. In addition, the recorder, being critically damped, has a retarded response to rapid changes in voltage (e.g., when the gages are strained rapidly).

Calibration against an oscillograph indicated that a maximum interval of 1.2 seconds could elapse between the instant of imposing strain and the travel of the pen to the exact site of a unidirectionally moving signal. The peak load recorded is a function of the "instantaneous" maximum load. With a typical fresh starch gel, when the peak load recorded was 73 g., the "instantaneous"[2] maximum load was 420 g.

Discussion

Stress relaxation is determined in a variety of ways, mostly in samples placed under traction. A common method is to measure stress by the use of a balance arm, to which the sample is attached (2, 15), or by the displacement of a calibrated elastic element (4, 12, 16). With the latter approach, stress relaxation in elastic sols has been measured by means of changes in the torsion of a twisted wire, from which a bob is suspended in the sol (9, 13). The amount of "recoil" or recovery of a deformed sample has also been taken to represent the amount of residual stress in a material at a given time (5, 12). Mooney *et al.* (11) have used the ingenious technique of determining the maximum resonance in vibration of stretched rubber samples, at various times, in order to ascertain the residual stress.

However, the methods mentioned above suffer from the discontinuity of observations, particularly in the early stages, when there are rapid changes in stress. To circumvent this difficulty, Hlynka and Anderson (10) and Cunningham *et al.* (8) have used a kymograph for

[2] This maximum occurred at 0.01 second after imposition of strain.

a continuous record of stress in stretched dough samples. In these cases, the stress is measured by the movement of the core of a differential transformer, which is attached by a spring and lever to one end of the sample. Continuous recording is also provided by Thorsen (14), in extending wool fibers, by attaching one end of the fiber to a special thin wire, which acts as a strain gage, i.e., its electrical resistance changes very sensitively with the strains imposed on it.

The use of two strain gages in the present apparatus confers some interesting advantages. As mentioned above, not only do they permit continuous recording of stress, but they also give a doubling of the output potential in being mounted on both sides of the cantilever beam. In addition, the gages are subjected to the same temperature; i.e., inasmuch as each comprises an arm of the Wheatstone bridge, resistance changes due to temperature will be identical in both arms (3).

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RELAXATION OF STRESS IN STARCH JELLY CANDY[1]

CLARENCE STERLING[2]

ABSTRACT

The rate of relaxation of stress in a starch confectionery jelly has been ascertained with a continuously recording instrument. One-day-old gel samples have been compressed to various degrees of strain for varying periods of time. Relaxation of the stress does not follow a Maxwellian curve (i.e., a curve for a simple Maxwell model). Limiting values of stress indicate a residual elastic response for which a modulus can be calculated. The time of relaxation and the ultimate stress attained vary with the amount of strain imposed. Elastic properties of the gel determine the value of the ultimate stress and of the stress at any particular time during the period of strain. The mechanical behavior of the starch gel does not follow the superposition principle. Molecular changes are interpreted in the light of experimental results.

When an ideally elastic material is subjected to a constant tensile or compressive strain, a corresponding stress arises in the material. As long as the strain is relatively small, the amount of induced stress will remain constant. If, however, an ideally viscous substance is subjected to strain, the stress produced by the straining of intermolecular bonds rapidly decreases as the molecules move past each other to take up less strained positions.

The term "relaxation time" has been advanced (16) to characterize the relative rate of decay of stress in any strained material. If the strain imposed on a body be held constant, the time of relaxation is that definite chronological interval necessary for the originally induced stress to sink to 1/e of its original value (where e is the base of the Napierian logarithm). In an elemental Maxwell body (1, 19, 21), in which a spring and a dashpot are combined in series,

$$(d\sigma/dt) = (1/\eta) \cdot S + (1/n) \cdot (dS/dt) \qquad (1)$$

where σ is the strain,
t is the time,
η is the coefficient of viscosity,
S is the stress, and
n is the shear modulus of elasticity.

At constant strain, $(d\sigma/dt) = 0$, hence

$$(1/n) \cdot (dS/dt) + (1/\eta) \cdot S = 0$$

$$(dS/S) = (n/\eta) \cdot dt$$

Integrating, $\ln \cdot (S/S_0) = -(n/\eta) \cdot t$, (2)

where S_0 is the original stress,

[1] Manuscript received October 29, 1956.
[2] Department of Food Technology, University of California, Davis, Calif.

S is the stress at any time, t,

and $S = S_0 e^{-(n/\eta)t}$ (3)

The expression, (n/η), has the dimension of a reciprocal time, and it is obvious that this value is the reciprocal of the "relaxation time" defined above.

With a "perfectly" elastic solid, the time of relaxation approaches infinity; with a "perfectly" viscous fluid, it is virtually instantaneous. Between these two limits occur the values for all real materials. Thus, for water the relaxation time has been estimated at 10^{-12} seconds, for molten glass at 10^{-7} to 10^{-4} seconds, for swollen gels at 10^{-4} to 10^{0} seconds, and for dry wool and cotton at 10^{1} to 10^{5} seconds (15). It may readily be seen that with a simplified model, the concept of relaxation time provides a valuable measure of the relative proportion of its viscous and elastic properties.

Previous studies (via the method of strain retardation or "creep" measurements) have revealed some aspects of the behavior of starch jelly candy (22, 23, 24). Results obtained from stress relaxation measurements should provide an interesting comparison with these earlier findings. They might also serve to elaborate further the molecular behavior of the deformed gel.

Materials and Methods

The composition and the method of preparation of the jelly have been described in another study (22). Also, the recording apparatus and the manner of manipulating the specimen were the subject of a previous report (5). In the present investigation, samples were taken at the age of 24 hours and subjected to compressive strains varying from 1.9% (0.5 mm. deformation) to 36.8% (8 mm. deformation) for periods of 20 minutes. (Sample size is 1.27 cm. diameter by 2.60 cm. length.) A separate sample was used for each determination.

Two variations of the manipulative technique were carried out at a strain of 26.2% (6 mm. deformation): 1) the rate of straining was varied from 0.1 second to several seconds; and 2) after 120 seconds of straining, the specimen was allowed to recover for 10 seconds and then subjected again to the original 26.2% strain.

Stress values were determined from the recorder curves on the basis of daily calibrations and on the assumption of a Poisson ratio of 0.5 (see 22).

Results and Discussion

The data of Table I are the averages of the values of stress at the various strains imposed. Some of these data are presented graphically

in Fig. 1. If a simple Maxwellian element were involved, there would be a rectilinear relationship between the time of strain and the logarithm of the stress (equation 2). Such a relationship has been found in but few polymeric gels (17, 20). Instead, some workers have recorded a rectilinear relationship between the stress and the logarithm of the time of strain (10, 18). For the most part, few attempts have been made to explain this type of relationship.

TABLE I

STRESS ($\times 10^3$ DYNES/CM.^{2}) DURING CONSTANT STRAIN

STRAIN	TIME (IN SECONDS)									
	1	5	10	20	40	60	120	300	600	1200
%										
1.9	16.1	6.1	4.7	4.0	3.4	3.2	2.9	2.6	2.6	2.6
3.9	28.0	12.2	10.8	9.4	8.4	7.7	7.2	6.7	6.4	6.1
8.0	30.0	13.7	11.8	10.5	9.5	8.9	8.3	7.4	6.7	6.2
12.3	32.6	16.7	14.8	13.2	12.3	11.6	10.7	9.9	9.3	8.8
16.7	36.4	22.2	20.7	19.0	17.9	17.3	16.4	15.4	14.6	13.9
21.4	44.5	27.8	26.9	25.0	23.6	22.6	21.6	20.6	19.6	18.9
26.2	43.5	31.3	29.0	27.0	25.5	24.7	23.6	22.3	21.3	20.6
31.4	51.8	39.5	36.9	34.9	32.8	32.0	30.3	29.0	27.6	26.8
36.8	62.4	48.0	46.7	43.9	41.6	40.4	38.8	36.8	35.6	34.5

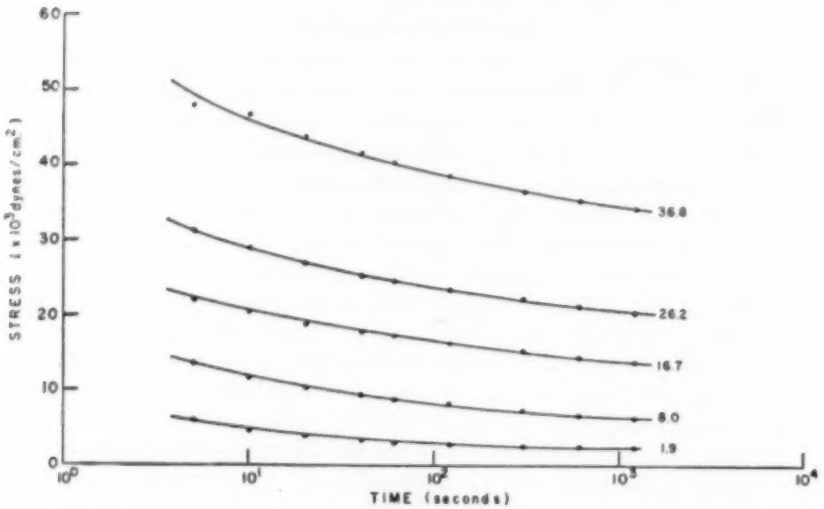

Fig. 1. Change of stress with time in a starch gel during constant compressive strain. The percentage of strain imposed is indicated at the right of each curve.

However, it is to be noted that the curves of Fig. 1 (stress vs. logarithm of time) are not rectilinear but curvilinear, tending to approach

a limiting value asymptotically – perhaps attained within 20 minutes at a strain of 1.9%. A similar result was found in polyisobutylene (3), rubber (25), and flour dough (6, 7, 11), but in these materials the ultimate value of the stress was zero. (Bohn and Bailey, 4, indicated a rectilinear log stress/log time relationship in flour dough.) Results more likely akin to those in the starch jelly were shown in raw rubber by Treloar (26). Attainment of an ultimate positive value of stress would indicate a residual elastic force (also 9). Assuming that the ultimate stress were reached at 20 minutes of strain, the average Young's modulus of this elasticity would be 9.8×10^4 dynes/cm.^{2} (The relationship between strain and residual stress at 20 minutes is rectilinear.) This value is quite close to that established earlier, on the basis of strain retardation determinations, as the intrinsic Young's modulus of the gel, 7.8×10^4 dynes/cm.^{2} (22).

For reasons advanced earlier (5), it is not feasible to consider that the initial peak stress value (Table I) corresponds closely to the true value of the starting stress. Nevertheless, if it be assumed that the recorded value approximates the peak stress on a proportionate basis, it is possible to estimate a "relaxation time," i.e., an amount of time in which the stress decays to 1/e of its initial value. This "relaxation time" increases slowly with the smaller strains and very rapidly and abruptly with strains over 16%. (Thus, it is 6 seconds at 1.9% strain, 52 seconds at 12.3% strain, and over 1200 seconds at 16.7% strain; see 17, 20, 26.)

Since this result, together with the non-Maxwellian course of the relaxation curve, negates the assumption of a simple Maxwell element, the only course open to a mechanical analogy is to consider the behavior as an integral of varying Maxwell (and perhaps Kelvin, 19) elements. In other words, a spectrum of relaxation times (1, 13) could be assigned to the jelly, each representing varying steps in its combined elastic and viscous properties.

Because a mechanical analogy must be referred ultimately to the molecular situation, it is appropriate to speculate directly about the latter. The existence of a residual stress, of virtually the same modulus, at all strains, would indicate that the elastic force is due to the same molecular situation in each case. Presumably the initial imposition of strain ruptures the majority of bonds which will be ruptured. Since the strain is not increased further, the remainder of the strained bonds probably tend to remain stabilized for the most part. Small strains may not produce any ruptures, the short "relaxation time" representing the viscous movement of molecules in the gel. With larger strains, perhaps in the neighborhood of 12–16%, following the ini-

tial bond rupture, there may occur a crystallization or retrogradation of parallel linear regions of the polysaccharide molecules (see also 22, 23).

Not only at the 1200-second value of stress but also at other time intervals, a rectilinear relationship between residual stress and the strain is found. Figure 2 shows this relationship at the 60-second period. When the slopes of such relationships are calculated on the

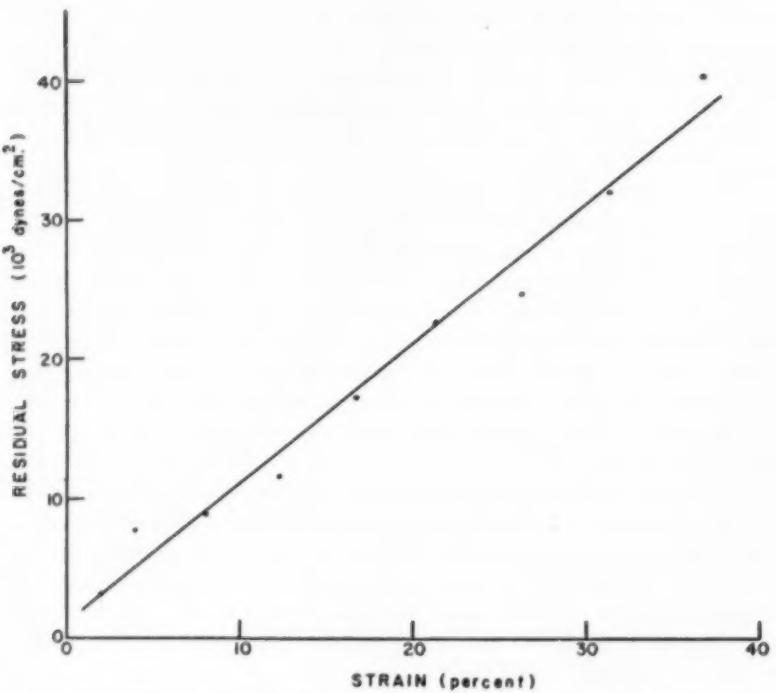

Fig. 2. Residual stress after 60 seconds, referred to the amount of constant strain imposed on the starch gel.

basis of the least squares method, the values of the "Young's modulus" thus obtained vary somewhat from those obtained on an arithmetic basis. If these values be plotted against the logarithm of time, they fall on a straight line (Fig. 3). Hence the modulus follows the relationship:

$$E = m \ln t + C, \tag{4}$$

where E is the Young's modulus of elasticity,
m is the slope of the line,

t is the time of strain, and
C is a constant.

(Note the resemblance to the effect of age on modulus in strain retardation (24).) Thus, the modulus may not reach a constant value at 1200 seconds but probably decreases continually, at a rate proportional to the logarithm of the time of strain.

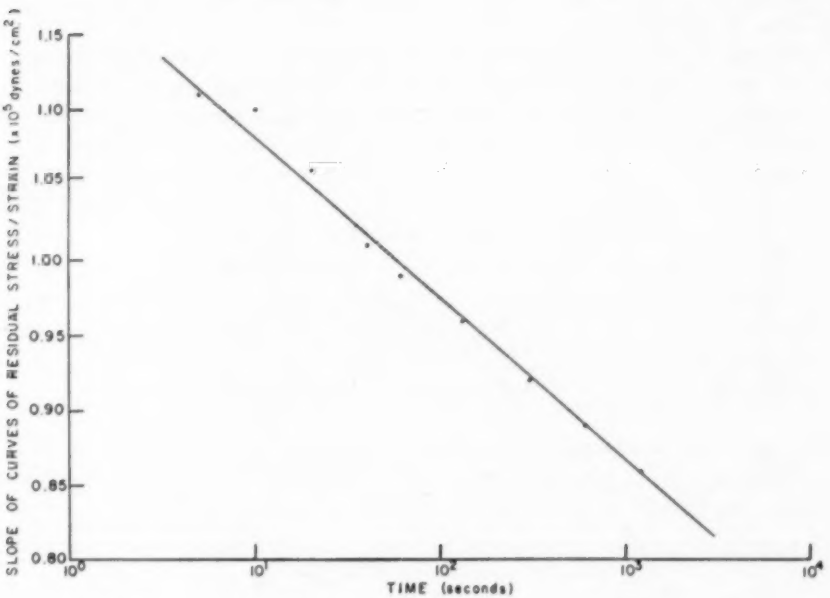

Fig. 3. Slopes of curves of residual stress/strain (like that of Fig. 2) for different periods of straining.

A decay in the modulus is shown also in the experiments in which the sample is permitted to recover after 120 seconds of strain and the strain then reimposed after 10 seconds. In all cases, the peak stress at the second straining is much lower than the initial peak stress. However, it must be recalled that there has already been bond rupture during the first straining, so that the lower peak stress is not due entirely to the decay in modulus which has taken place in the first 120 seconds of strain.

Another aspect of the peak stress is of interest. When the strain is imposed slowly, the peak stress is much higher than when the strain is imposed rapidly. Since it is to be expected that the opposite effect

should obtain,[3] this finding is of some interest. It is explicable on the basis of assuming either that some type of reinforcement of bonds occurs or that a less rapid "impact" takes place with slower straining, leading to lesser rupture of the network bonds. Perhaps the slower strain can be more readily distributed over all the intermolecular junctions rather than being concentrated in a portion of them. In any event, it is apparent that the properties of the gel are principally properties of an elastic network. The viscous qualities play only a minor role in its behavior.

Despite the essentially elastic properties of the molecular system of the gel, it should be recognized that the linkages are weak ones. Consequently, the gel is readily disrupted, and apparently, its be-

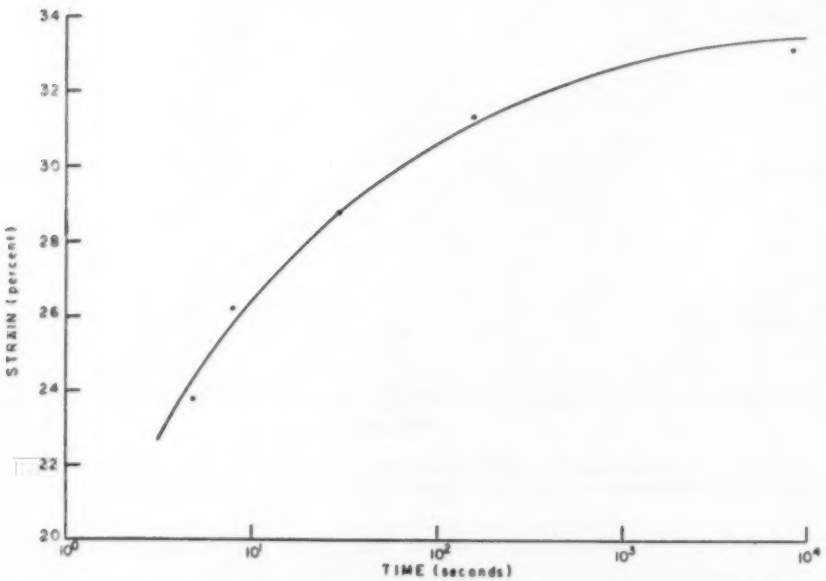

Fig. 4. Interpolated strain/time values for a stress of 30×10^3 dynes/cm.^{2}

havior does not follow the superposition principle (14). Thus, it is not possible to transpose directly the results on relaxation of stress with those on retardation of strain. In this connection, it is of interest to compare Fig. 4 here with Fig. 5 (50-g. loading creep curve) of the earlier study (22). Figure 4 is an interpolation from Fig. 1, projecting

[3] A rapid rate of straining is more likely to induce a measurable stress because the time of straining can be less than the time of dissipation of the stress. Note, for example, that only with a rapid rate of straining is it possible to obtain a dynamic elastic modulus for a liquid (8, 12).

the stress of 30×10^3 dynes/cm.^{2} in terms of its strain/log time effects. It is thus fairly comparable to the 50-g. loading of the earlier study. Note that there are considerable differences in the magnitudes of the strain values and a general difference in the over-all shape of the curve. Alfrey and Doty (2) have indicated that creep functions and relaxation functions can be interrelated in a material which is viscoelastic (i.e., follows the superposition principle).

As a final observation, it may be pointed out that the relaxation curves of the present study are somewhat more dependent on the initial peak stress than on the absolute value of the strain. If the initial stress is higher, the stress values for the rest of the period are higher, regardless of the strain imposed. Again, this finding shows the dependence of the stress relaxation values on the elastic properties of the polysaccharide network.

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FREE PENTOSES IN MALT EXTRACTS AND WORTS[1]

W. O. S. Meredith

ABSTRACT

The presence of free arabinose and xylose in malts and extracts and worts has been confirmed by chromatographic procedures followed by preparation of crystalline derivatives by the micro methods of White and Secor. Free xylose was about 0.3% of total sugars of worts of commercial six-row barley malts and from 0.8% to 2.5% of total sugars of worts of experimental malts of pure six-row varieties. Cold-water extract was relatively higher in pentose content than laboratory worts, which suggests that pentosan degradation during malting may be a key process.

During studies on malt gums in this laboratory, free pentoses were detected in malt extracts by chromatographic procedures (2, 3). The pentoses probably arise mainly from the pentosan component of the mixture of barley gum polysaccharides which are assumed to be related to cell-wall material, and which have also been shown to contain hexosans (7, 16, 19). Hexoses, on the other hand, may arise from either starch or gums. The pentoses may thus be considered as key substances in the broader study of the biochemistry of malting, of which the study of gum degradation during malting and mashing is a part.

[1] Manuscript received September 20, 1956. Paper No. 159 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, and No. 339 of the Associate Committee on Grain Research (Canada).

Evidence for the presence of free pentoses in malt and wort has been conflicting. McLeod, Travis, and Wreay (15) indicate that free pentoses are produced during malting but that they disappear again, so that malt contains no free pentoses. Preece and Aitken (19) suggest that appearance of free pentoses in malt and wort is not normal. Barton-Wright and Harris (4) also indicate that worts do not contain free pentoses. Harris, Hall, and MacWilliam did not detect free pentoses in malt in one study (12) but in a later study Hall, Harris, and MacWilliam (11) detected them in small amounts. Similarly, Gjertsen recently presented evidence for free pentoses in malt (9), whereas previously he had not detected them (8). McFarlane and Held in 1953 indicated absence of pentoses in worts (14), but since then they have indicated that they are present.[2] Montreuil and Scriban (18) detected pentoses in malt, wort, and beer, and Green and Stone (10) detected pentoses in fermenting beer.

Many of the differences in claims for or against the presence of free pentoses in wort may arise from the incomplete chromatographic separation by some solvents of arabinose from fructose and mannose in complex mixtures such as found in malt extracts. Secor and White (21) faced similar difficulties and resolved them by means of their elegant hanging-drop technique for differential crystallization of arabinose in mixtures. This paper presents results of quantitative measurements of free xylose in six-row barley malts and worts, and confirmation of the presence of free arabinose and xylose by means of the microcrystallization procedure of Secor and White (21) and of White and Secor (22).

Materials and Methods

Four samples of typical commercial six-row barley malts and four brewhouse worts were obtained locally for examination, along with a number of experimental malts prepared from samples of pure six-row barley varieties. The malts were ground in a fine-grind Miag mill and extracted by various procedures to provide liquors for chromatography. The procedures were: (a) extraction under reflux with boiling 85% ethyl alcohol for 30 minutes; (b) cold-water extraction at 20°C. for 2 hours; (c) the A.S.B.C. (Congress) extraction procedure (1), 30 minutes at 45°C., raise to 70°C. in 25 minutes, hold at 70°C. for 60 minutes; and (d) extraction with water at 70°C. for 2 hours. Brewhouse worts were chromatographed directly without further treatment.

The qualitative chromatographic procedures were similar to those used previously (16), using Whatman No. 1 filter paper and the following developing solvents: (A) ethyl acetate; pyridine; water, 5:2:5. (B) ethyl acetate; acetic acid; water, 3:1:3. (C) 1-Butanol; acetic acid;

[2] Personal communication.

water, 4:1:5. Solvent A was used regularly and solvents B and C were used to check identification of the sugars. Aniline phosphate was used as a spray to locate the position of the sugars (5). Hexoses and disaccharides produced shades of brown and pentoses produced a clear pink color. Fructose was detected by the naphthoresorcinol spray (13).

Preparation and concentration of the pentoses was carried out chromatographically on Whatman 3 MM paper. Liquors were streaked on a thin line along the central 4-in. section of a 6-in. paper 22 in. long. Check solutions of glucose, arabinose, and xylose were spotted on the outer 1-in. section. After development by solvent, the papers were dried and the outside 1½-in. sections were cut off and sprayed. The outer edges were matched with the central portion and the areas containing the pentoses were cut out. These were eluted with 50% ethyl alcohol and the eluates were concentrated under reduced pressure.

Quantitative measurements of pentoses and other sugars in the various extracts were made by the procedures of Dubois, Gilles, Hamilton, Rebers, and Smith (6), using solvent A as the developing reagent for sugar separation.

The eluates were checked chromatographically for presence of sugars and were used to provide crystalline derivatives of arabinose and xylose by means of the hanging-drop techniques of Secor and White (21), and White and Secor (22). Arabinose was checked as the 2:4 dinitrophenylhydrazone-pyridine-solvate, and xylose was checked as the phenylosazone.

Results

Quantitative Measurements. All the extracts and worts contained a wide variety of sugars and oligosaccharides. Arabinose and xylose were readily detected by the pink color produced on the chromatograms by the aniline phosphate spray. The amounts of the individual oligosaccharides, disaccharides, and monosaccharides were determined (6). Since the chromatograms showed that xylose was separated completely from the other sugars, whereas arabinose and fructose overlapped, the ratio of xylose to the sum of the carbohydrates in the various extracts was calculated (see Table I).

Confirmation of Pentoses. Arabinose was crystallized as the 2,4-dinitrophenylhydrazone-pyridine solvate by the method of Secor and White (21). The crystals from pure arabinose and those from arabinose obtained by chromatographic separation from the sugar mixtures were identical under the microscope, and similar to those illustrated by these workers.

Identification of xylose as the phenylosazone by the method of

TABLE I
DATA FOR MALT LIQUORS AND MALT

MALTS	NO. OF SAMPLES	TOTAL SOLUBLES	XYLOSE, AS PERCENT TOTAL SUGARS	XYLOSE, AS PERCENT MALT
		%	*range*	*range*
Commercial				
Congress wort	4	75	0.30–0.35	0.22–0.26
Brewhouse wort	4	...	0.20–0.28	...
Experimental				
Alcohol extract of malt	8	12	0.96–2.2	0.11–0.26
Congress wort	8	75	0.80–2.5	0.60–1.9
Hot-water extract	8	75	0.80–2.5	0.60–1.9
Cold-water extract	8	20	3.4 –5.0	0.68–1.0

White and Secor (22) presented no difficulty when pure xylose was used, but the xylose isolated from worts by chromatography failed to give a crystalline derivative. However, with the use of larger amounts of the xylose solution and the phenylhydrazine reagent, and heating of the mixture for a few minutes at 100°C., the phenylosazone crystallized after 3 days in the characteristic form of scattered yellow needles.

Discussion

Only four commercial six-row barley malts and four brewhouse worts were examined, so that only limited conclusions on characteristics can be made. However, free pentoses were present in the liquors in relatively small amounts. The brewhouse worts obtained from mashes containing adjuncts contained less xylose than the Congress worts.

The ranges for the xylose content of solubles and of grain from experimental malts of pure six-row varieties appear relatively great, but quite large differences between varieties in other properties are quite common (17 and preceding papers in the series). The values for xylose content as percent of malt extracted by the different extraction procedures gives some indication of the extent of pentosan degradation occurring during malting and mashing. The Congress worts and the hot-water worts contain about the same amount of total extract and the same amount of xylose, and the ratio of xylose to total solubles is similar to that for the alcohol extract. The values for cold-water extract present a different picture. The percentage of xylose in the solubles is high, and the amount of xylose extracted from the malt is about equal to that obtained by the higher temperature extractions, and exceeds that extracted from the malt by alcohol.

Without the data for the alcoholic extracts, the inference might be that, as barley contains only small amounts of free pentoses (11), pentosan degradation to free sugars occurs mainly during the malting

process. As alcohol extracts only a small amount of xylose from malt, extensive degradation of pentosan to free xylose during malting is not indicated. Nevertheless, it is clear that xylose is readily extracted, or produced during low temperature extraction of malt with water. Extraction at higher temperatures does not produce much more xylose. There is thus a general similarity between characteristics of pentosan material and protein material, as it is generally recognized that about 75% of nitrogenous material extracted from malt by the Congress procedure is extractable at 20°C., even under conditions of enzyme inhibition. On the other hand, only about 25% of the carbohydrate material extracted by the Congress procedure is extracted at 20°C. even when the enzymes are active during extraction. The preferential release of free xylose as compared to hexose during mashing at 20°C. may arise from either, or even both, of two conditions: the pentosans may be more extensively degraded than hexosans during malting, so that the former are readily hydrolyzed at low temperatures; or the pentosanases may have lower optimum temperature requirements than the hexosanases. All aspects of pentosan degradation and pentosanase activity require further study. In the meantime, our results tend to confirm the hypothesis that pentosan material, related to cell-wall constituents, is degraded during malting in order to release starch which is hydrolyzed during subsequent mashing of the malt. Thus pentosan degradation may be a key process in malt modification.

Major difficulties in the study of pentosan degradation have been encountered in detecting degradation products among relatively high amounts of hexose residues in *in vivo* studies, and the lack of suitable substrates for *in vitro* studies. Chromatography has assisted the *in vivo* studies, and *in vitro* studies are now being carried out by Preece and Hobkirk (20), who have used an arabo-xylan from rye as a substrate for measurement of pentosanase activity of barley. The problems in the study of barley pentosan degradation and its importance in malting are therefore becoming more amenable to attack. The confirmation discussed above of the presence of free arabinose and free xylose in six-row barley malt may have some bearing on the elucidation of pentosan degradation.

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THE INFLUENCE OF THE WATER-SOLUBLE CONSTITUENTS OF WHEAT FLOUR ON ITS MIXING AND BAKING CHARACTERISTICS[1]

PAUL J. MATTERN AND R. M. SANDSTEDT

ABSTRACT

The principal factor responsible for determining the mixing requirement of wheat flour is water-soluble. The mixing time of a flour can be lengthened by removal of the water-solubles; reincorporation of the solubles reverses this effect. The factor is precipitated by low concentrations of ammonium sulfate, and has a high amide nitrogen content indicating that it largely consists of gliadin. Gliadin prepared by the usual methods is an effective agent for shortening the mixing requirement of a flour. The mixing requirement of a flour may be extended by incorporating water-extracted flour with the normal flour.

For some years there has been a difference of opinion among laboratories concerning the importance of flour solubles to baking characteristics. Using doughs reconstituted from gluten and starch, Sandstedt, Jolitz, and Blish (19) observed that the baking properties were similar to those of the original flour. Using similar techniques Prentice, Cuendet, and Geddes (18) and Pence, Elder, and Mecham (14) have been unable to get good baking results without the addition of the water-solubles. Finney (5) found that the solubles were necessary with certain types of wheat and unnecessary with another. However, Harris and Sibbitt (7) and Bechtel and Meisner (3) have had no such difficulty. Such contradictory results indicate differences in methods of gluten washing and in baking procedures. Interpretation of the importance of the water-solubles from the results obtained from a starch-gluten reconstitution method is unreliable, inasmuch as the washed gluten is a mixture or complex which may vary widely depending on the methods and conditions of washing the gluten, and the consequent quantities of soluble components left in the gluten.

The work presented here stemmed from our desire to explain the differences found in the above-mentioned research; the work reported is primarily concerned with the effects of the water-soluble components on mixing requirements. A method of reconstitution, consisting of removing the water-solubles from the flour and then recombining fractions of these solubles with the residual extracted flour, made it possible to follow effects of the solubles on mixing properties of wheat flour.

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In recent years the baking industry has been placing increasingly greater emphasis on the mixing requirements of flour. Control of this factor would be of great value, not only to the baker, but also to the milling industry and to wheat breeders.

Materials and Methods

Flours. The water-extracted flour samples were prepared from nonbleached, nonmalted straight-grade flours, unless otherwise noted.

1. 1952 Nebraska hard winter wheat composite, milled on the Kansas State College mill. Protein 11.50% (14% M.B.).
2. 1954 Nebraska hard winter wheat composite, milled on the Kansas State College mill. Protein 12.30% (14% M.B.).
3. A commercial nonmalted hard spring wheat flour. Protein 13.40% (14% M.B.).
4. A commercial bakery flour consisting of a blend of hard spring and winter wheats, bleached and malted. Protein 12.90% (14% M.B.).
5. Hard winter wheat, 1½ minutes farinograph mixing time (500 B.U. maximum consistency), experimentally milled. Protein 10.85% (14% M.B.).
6. Hard winter wheat, 15 minutes farinograph mixing time (500 B.U. maximum consistency), experimentally milled. Protein 11.50% (14% M.B.).

Preparation of Extracts. One part of flour was suspended in 10 parts of distilled water and stirred continuously for 1 hour. The suspension was then allowed to settle and the supernatant was centrifuged. Additional treatment such as dialysis, heating, or enzyme treatment will be mentioned as the experimental results are reported.

Preparation of Water-Extracted Flour. The residual flour from the above extraction was treated with a second portion of water equal to the volume removed, and allowed to settle. The supernatant liquid was removed and combined with the first extract. The residual flour was either lyophilized or air-dried on a large glass plate. The lyophilized sample was ready for immediate use, whereas the air-dried sample was coarsely ground in a burr-type mill and then reduced on the smooth rolls of an Allis Chalmers experimental mill. Excessive damage to the starch was avoided by conditioning the coarsely ground material to 15–16% moisture in a humidity box before reduction on the smooth rolls. The lyophilized flour was easier to handle and gave more consistent mixing times.

Proteolytic Enzyme Treatment. Boiled 1:10 flour extracts were

given a 48-hour digestion with ficin or with papain (25 mg. enzyme per 100 ml. of extract). This amount of papain (8) is three times as much as is generally required to overcome the papain inhibitor of the extract. After digestion the samples were again boiled, dialyzed, and lyophilized.

Pentosan Preparations. Preparations of soluble pentosans were made according to the procedures of Pence, Elder, and Mecham (13), Baker, Parker, and Mize (2), Perlin (16), Meredith, Watts, and Anderson (11), and Simpson (20). Fractionated pentosans were prepared by Perlin's (17) procedure, modified, however, in that the fractions were merely separated and not subjected to complete hydrolysis.

Gliadin Preparation. Gliadin was extracted with 0.01 *N* acetic acid from washed gluten which had been dried at 60°C. in a vacuum oven (4). (Drying the washed gluten on plates covered with Saran Wrap completely eliminates the adhesion of the dried gluten to its container). After the solution was centrifuged, the gliadin was precipitated with 5% sodium chloride, dissolved in 70% ethyl alcohol (by weight), reprecipitated with 95% ethyl alcohol, and dried with absolute ethyl alcohol, acetone, and ethyl ether. Gliadin analysis: 90.2% protein (N × 5.7) : 28.4% amide N.

Ammonium Sulfate Fractionations. Fractionations of the water-soluble extracts were made by precipitation with ammonium sulfate, using the ammonium sulfate concentrations recommended by Pence and Elder (12).

Mixing Curves. Since mixograms and farinograms do not necessarily agree, mixing curves were run on both the National Micro-Recording Mixer and the Brabender Farinograph. The mixograms were run on doughs made from 25 g. of flour (14% M.B.) at 25°C. The farinograms were on 50 g. of flour (14% M.B.) run at 30°C. When used in reconstituted doughs, dried flour extracts or gliadin replaced equal weights of the water-extracted flour.

Types of Doughs Used for Mixing Curves.

1. Yeastless
   a. Normal flour-water mixes
   b. 3-Hour soaker doughs – flour and water mixed with a spatula to incorporate the water and allowed to stand 3 hours before the final mix.
2. Yeasted
   a. Straight dough
   b. 3-Hour 100% sponge doughs – given a minimum first mix to incorporate the ingredients.

Dough Formula. Yeast 3%, sugar 7%, salt 1.5%, 25 g. flour (14% M.B.).

Analyses. The nitrogen content of extracts and water-soluble fractions was determined by the Gunning-Kjeldahl method. Pentosan analyses were run by conversion to furfural by the method of Jayme and Sarten (9) followed by precipitation of the furfural with 2,4-dinitrophenylhydrazine, drying, and weighing.

Amide nitrogen was determined by the method of Mecham and Olcott (10).

Results

Mixing Characteristics of Extracted Flours. Curves A and B of Fig. 1 are the control curves for the spring wheat flour (No. 3). The flour used for curve B was moistened, dried, and reground in order to simulate all treatments given the extracted flour, except the removal of the solubles. The soaking accompanying this treatment decreased the mixing requirement from 3 minutes to 1½ minutes. Curves C and D, Fig. 1, indicate that the removal of the solubles lengthened the mixing time to about 18 minutes in comparison to 1½ minutes for the control flour (B). The mixing time was longer as measured at the beginning of the fermentation period (C) than on the sponge dough after 3 hours of fermentation (D). Similar comparisons made on different flour samples, which appear later in the paper, always show the sponge (or soaker) dough with a shorter mixing time than the normal straight dough.

Before an evaluation the curves in Fig. 1 require some explanation. The narrow portion, at the beginning of the mixogram curves obtained from water-extracted flours, did not result from excessive addition of water but indicates that the weak cohesive forces in the dough did not allow the particles to stick together to form a gluten as in a normal flour. During this period the dough was not extensible and was not capable of holding gas. This is demonstrated again later in the paper with a comparison of duplicate runs on the farinograph and mixograph (Figs. 5 and 6).

One outstanding characteristic of extracted flours was their ability to withstand overmixing (Fig. 1), which again indicates a relation between solubles and extensibility and between solubles and the tendency for normal doughs to become exceedingly sticky and soft upon overmixing.

Effects of Adding Back the Solubles to Extracted Flours. The reincorporation of the lyophilized extract to this water-extracted flour (No. 3) allowed the rapid formation of an extensible dough; accordingly the mixing time was shortened to 4 minutes (Fig. 1, curve E).

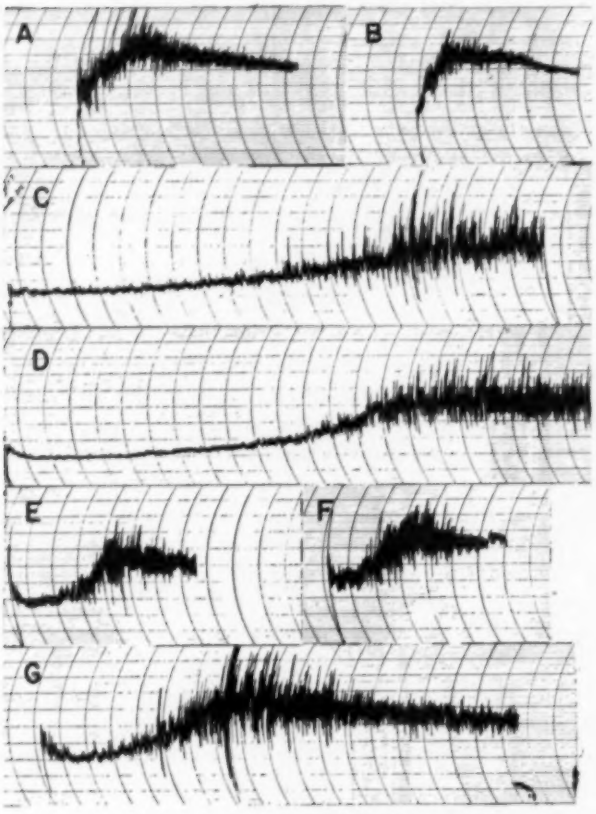

Fig. 1. Mixograms of Nebraska hard winter wheat flour (No. 1). A, original untreated flour; B, moistened, dried, and reground (no solubles removed); C, water-extracted flour, straight dough; D, water-extracted flour, sponge dough; E, water-extracted flour, plus total water-solubles, sponge dough; F, water-extracted flour, plus ficin-treated total water solubles, sponge dough; G, water-extracted flour plus 2½% pentosan preparation, sponge dough.

The fraction responsible for shortening the mixing time proved to be nondialyzable and heat-stable (boiling for 10 minutes).

Extracts also were treated with ficin and papain (and then boiled to destroy the enzyme) to observe any changes in effectiveness of the extract due to removal of the protein. The dough for curve F containing the boiled ficin-treated extract had a mixing requirement comparable with that of the dough containing the untreated extract (curve E). Papain treatment produced similar results. However, an analysis for nonprecipitable nitrogen (hydrolyzed protein) on papain- and ficin-

treated flour extracts showed that the water-soluble protein in the flour extract was exceedingly resistant to attack by these proteolytic enzymes under the conditions employed. Consequently the protein was not changed and curve F merely indicates that the enzyme and boiling treatment did not change the effectiveness of the extract.

Effects of Pentosans. Approximately 10% of a flour is water-soluble; of this about 10% is pentosan. The total pentosan contents of flours vary from about 2.25 to possibly 2.80%; about 40% of this is water-soluble. Accordingly the pentosans are among the possible effective agents. Adding 2.5% of a pentosan preparation (made by the procedure of Baker, Parker, and Mize (2)) to the extracted spring-wheat flour reduced the mixing time considerably (Fig. 1, curve G); however, not as much as the total extract (curve E). This pentosan preparation contained 64% pentosan; accordingly 0.4 g. of pentosan was added to the extracted flour as compared with 0.25 g. added in the total extract. Pentosans prepared by various other methods (listed under "Methods") produced similar results when added at 2–3% (on the flour basis). Fractionated pentosan preparations were less effective, particularly those fractionated with ethanol. These results indicate that the pentosan itself may not have been effective but that the preparations contained some other factor that was effective.

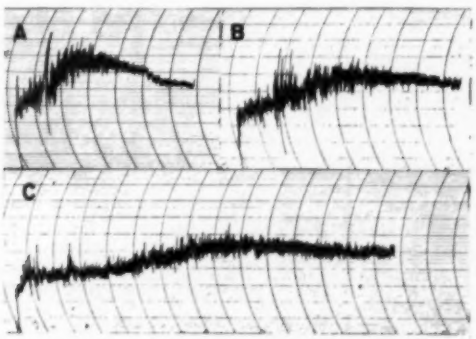

Fig. 2. Mixograms of Nebraska hard winter wheat flour (No. 1). A, untreated flour; B, 25% water-extracted, 75% untreated; C, 50% water-extracted, 50% untreated.

Mixtures of Extracted Flours with Normal Flours. Curves B and C of Fig. 2 show the mixing characteristics of blends of 25% and 50% extracted flour respectively with a normal flour (No. 1) whose curve is shown as curve A. This series of curves demonstrates that the mixing requirement can be varied over almost any reasonable range by the use of an extracted flour. If the water-extracted flour was lyophilized,

the baking properties of sponge doughs were only slightly impaired.

Extracts from Long- and Short-Mixing Flours. The data of Fig. 1 indicated that the removal of the water-soluble extracts had substantially changed the mixing requirements, and since natural flours have a variety of mixing requirements, it would appear possible that there would be differences in the composition of the extracts from two flours with widely differing mixing properties. Therefore, comparable extracts from a flour (No. 6) requiring a long mix (15 minutes farinogram) and one (No. 5) requiring a short mix (1½ minutes farinogram)

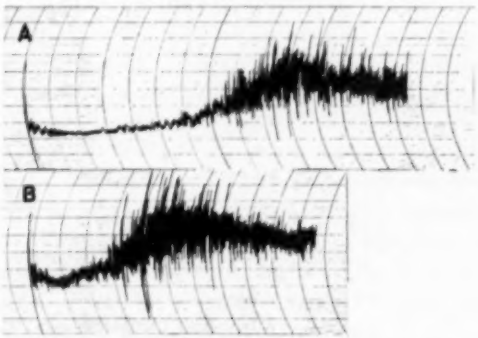

Fig. 3. Mixograms of water-extracted spring wheat flour (No. 3) using 100% sponge doughs with 3-hour fermentation. A, with extract from long-mixing flour; B, with extract from short-mixing flour.

were added to the water-extracted spring-wheat flour (No. 3). Curves A and B of Fig. 3 indicate that the solubles from a flour with a short-mixing requirement are more effective in reducing the mixing requirement than those from a long-mixing variety.

The following analyses point out a fundamental difference in soluble protein and amide nitrogen values between the extracts of short- and long-mixing wheat flours. The extract from the short-mixing flour had not only more soluble protein but also a higher amide N value.

Original flour protein (14% M.B.)	*Farinograph mix time*	*Average weight of extract from 25 g. flour (dry basis)*	*Protein in dry extract (dry basis)*	*Amide N, percent of total N (in extract)*	*Water-soluble protein, percent of total protein of flour*
	minutes	*g.*	*%*		
10.84	1½	2.2	29.6	18.8	20.6
11.52	15	1.9	26.8	15.7	15.2

This has been further verified as shown below by analyses of pairs of short- and long-mixing varieties containing the same amounts of flour protein.

Flour protein (14% M.B.)	*Farinograph mix time*	*Percent of flour extracted as protein*	*Water-soluble protein, percent of total protein*
	minutes		
12.85	4.0	2.28	15.3
12.85	9.5	2.07	13.9
12.30	4.5	2.34	16.4
12.30	10.0	2.00	14.0
11.00	4.0	2.34	18.3
11.00	9.5	1.97	15.4

Fractionations of the Water-Soluble Extract. The water-soluble extract from a 1954 Nebraska composite sample (No. 2) was fractionated with ammonium sulfate in an endeavor to obtain a fraction containing the component which would restore the normal mixing requirement to water-extracted flours. The fractions obtained were dialyzed and lyophilized.

Ammonium sulfate	*Protein in dry precipitate*	*N as amide N*	*Pentosan*
	%	%	%
0.4 M	70.6	20.8	23.1
1.74 M	86.6	16.6	13.5
3.65 M	40.5	9.2	48.8

These fractions were added to the extracted 1954 Nebraska flour (No. 2) at 3% levels. The fraction precipitated with 0.4 M ammonium sulfate was the most effective, the 1.74 M fraction second best, and the last fraction was comparable to the pentosan preparations tried earlier. Figure 4 shows part of the curves for this series. The fractions used in

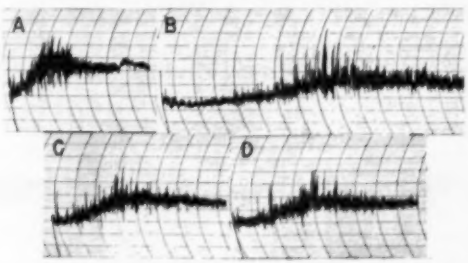

Fig. 4. Mixograms of Nebraska hard winter wheat flour (No. 2) 100% sponge doughs. A, untreated flour; B, water-extracted flour; C, water-extracted flour plus 3% of heated 0.4 M ammonium sulfate-precipitated fraction of water-solubles; D, water-extracted flour plus 3% of heated 1.74% ammonium sulfate-precipitated fraction of flour water-solubles.

the doughs for the curves shown were boiled for 10 minutes; similar curves were obtained by the use of the nonheated extracts. Apparently the fraction responsible for shortening the mixing requirement was heat-stable (at a pH of 6.5 as used).

Pentosan preparations are usually found to be contaminated with protein material (20) and, conversely, fractions of the water-soluble proteins contain pentosan impurities (12, 13). It is reported that the pentosan content of the albumin fraction (1.74 M ammonium sulfate

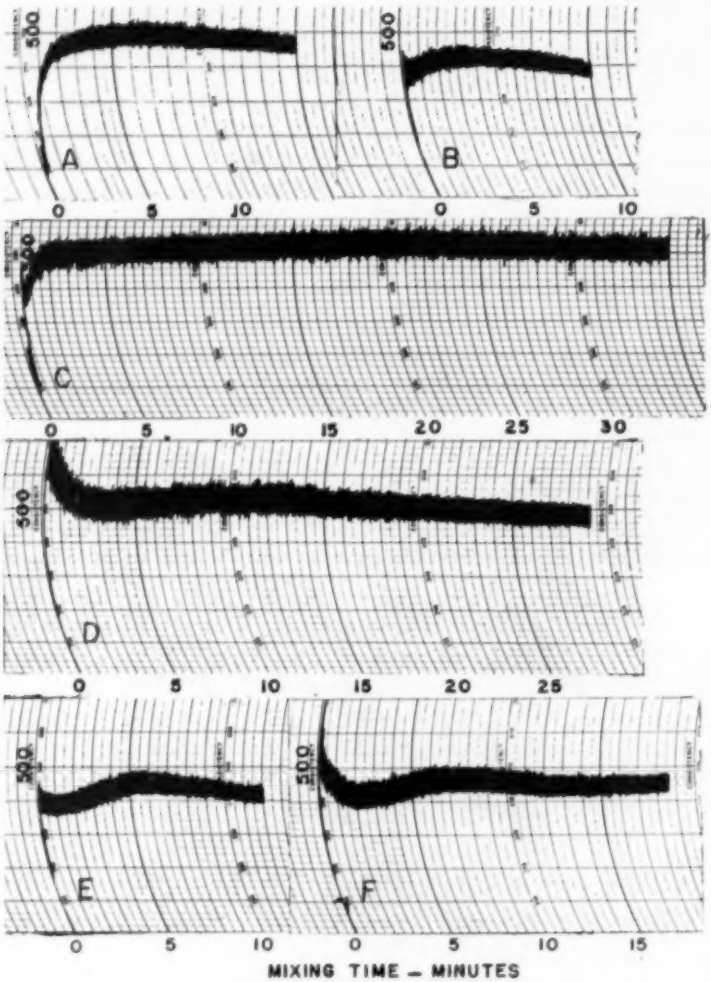

Fig. 5. Farinograms of a bakery flour (No. 4). A, untreated flour (59.8% absorption); B, untreated flour, 3-hour soaker dough (59.8% absorption); C, water-extracted flour (59.2% absorption); D, water-extracted flour, 3-hour soaker dough (59.2% absorption); E, water-extracted flour plus total solubles, 3-hour soaker dough (57.0% absorption); F, water-extracted flour plus 2% gliadin, 3-hour soaker dough (58.5% absorption).

precipitate) must be below 10% in order to get resolution of the albumin components on paper electrophoresis (12). These observations indicate the existence of complexes between the proteins and pentosans.

Gliadin and Mixing Requirements. Inasmuch as the pentosans were not completely effective in restoring mixing properties and the most effective fractions of the solubles had high protein contents, it seemed that the protein portion might be the factor responsible for controlling the mixing requirement.

Pence and Elder (12) pointed out that the high amide nitrogen values on the 0.4 M and 1.74 M ammonium sulfate fractions were due to significant amounts of gliadin. The amide nitrogen values as reported above also indicate the presence of gliadin in the fractions which were most effective in restoring near-normal mixing requirements.

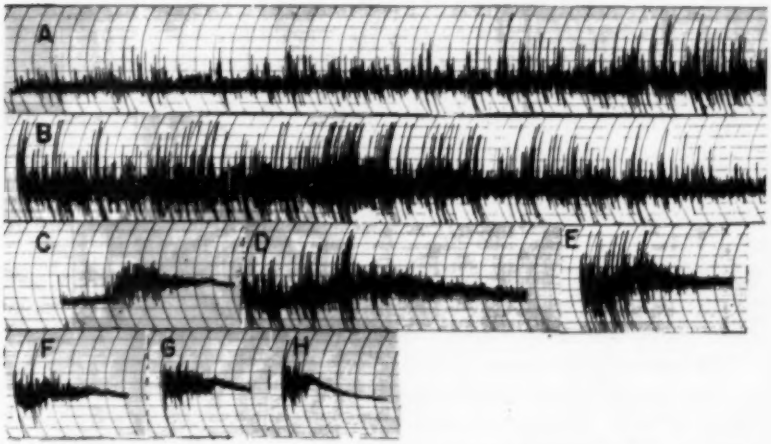

Fig. 6. Mixograms of a bakery flour (No. 4). A, water-extracted flour; B, water-extracted flour, 3-hour soaker dough; C, water-extracted flour plus total solubles, 3-hour soaker dough; D, water-extracted flour plus 2% gliadin, 3-hour soaker dough; E, water-extracted flour plus 4% gliadin, 3-hour soaker dough; F, water-extracted flour plus 6% gliadin, 3-hour soaker dough; G, water-extracted flour plus 8% gliadin, 3-hour soaker dough; H, water-extracted flour plus 12% gliadin, 3-hour soaker dough.

Figures 5 and 6 demonstrate the effect of gliadin on the mixing requirement of a water-extracted bakery flour (No. 4) as indicated by both the farinograph and mixograph curves, respectively. Curve A of Fig. 5 is the normal control curve of the original flour. Curve B is for a 3-hour soaker dough using untreated flour. Removing the water-solubles from the flour (lyophilizing the residue) extended the mixing requirement from about 7 to 22 minutes as shown in curve C. Curve D

shows that soaking the water-extracted flour reduced the mixing time from 22 to 13 minutes. The effect of total reconstitution by combining the water-extracted flour with its total solubles is shown in curve E. Replacing the total solubles with 2% gliadin produced a mix time of 7½ minutes which compares favorably with the 6-minute mix time with total solubles.

Absorptions on the soaker doughs were determined by first running nonsoaker duplicates at a consistency of 500 on the farinograph. The consistency fell below 500 on the soaker dough in Fig. 5, with B the control, E the total reconstituted dough, and F the water-extracted flour combined with 2% gliadin. Although it must be kept in mind that soaker doughs produce a different type of curve than the usual, a comparison of curve D with E and F indicates that the total solubles and 2% gliadin were instrumental in shortening the mixing requirements.

The absorptions used for the mixograms in Fig. 6 were identical with those used for the farinograms in Fig. 5. Curves A and B (Fig. 6) are the normal and soaker mixograms, respectively, of the lyophilized water-extracted flour. A mixing curve of this type in reality has no meaning since it is merely an indication that the materials making up the mixture are relatively inert toward mixing; the mixture has little extensibility and accordingly it demonstrates that unless the particles form an extensible medium a real mixing curve does not result. (Perhaps this is the difference between long and short mixing requirements.) It should be noted that the farinograph curve C of Fig. 5 appears perfectly normal; it does not reflect the inert nature of the water-extracted flour and accordingly may be misleading.

The absorption required to give a 500 reading on the farinograph seems to be quite generally accepted as a standard absorption for farinograph work. The absorptions used for making the mixograms of Fig. 6 are those required to give the 500 farinograph reading. However, the authors do not think that these are the correct absorptions, particularly in the case of the water-extracted flours.

The data of curve C indicate that the mixing characteristics of the original flour may be restored to an extracted flour if the solubles are reincorporated in the dough. In curves D, E, F, G, and H, gliadin has been incorporated with the water-extracted flour at levels of 2, 4, 6, 8, and 12% (of the total mixture), respectively. About 4% gliadin was necessary to produce a curve which indicated a mixing requirement similar to that resulting from total reconstitution. Curves F, G, and H would seem to indicate the need for only enough mixing to incorporate ingredients. It is significant that shorter mixing requirements seem to be accompanied by loss of tolerance to overmixing.

Effects of Reduction and Oxidation. The fact that reducing agents can be employed to shorten apparent mixing requirements of normal flours is well known. In the case of a water-extracted flour, 8 mg.% of cysteine gave substantial shortening of the mixing time. With water-extracted flours some variability would be expected depending on the amount of oxidation occurring during the extraction treatment.

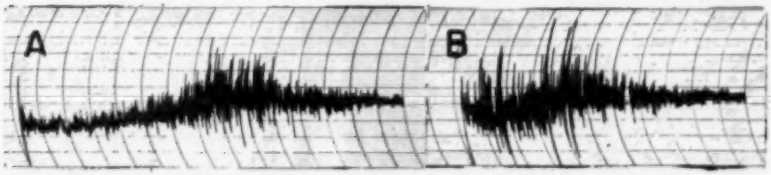

Fig. 7. Mixograms of 3-hour soaker doughs of water-extracted bakery flour (No. 4) with 5 mg.% potassium iodate. A, plus total solubles; B, plus 6% gliadin.

Adding 5 mg.% of potassium iodate to a total reconstituted water-extracted dough (flour No. 4) at the beginning of the 3-hour soaking period extended the mixing requirement about 4 minutes (curve A, Fig. 7). The control curve is curve C, Fig. 6. Likewise, 5 mg.% of potassium iodate added to a water-extracted flour containing 6% gliadin extended the mixing time 2 minutes (curve B, Fig. 7). The control is curve E, Fig. 6.

In both instances the original mixing requirement of the control was doubled. Such drastic changes in mixing times are not observed when oxidizing agents are added to normal flours, perhaps because the complexing existing between the gluten proteins is so great that the oxidizing agents are not as effective as when added to the soluble components before complexing occurs.

Baking Properties of Water-Extracted Flour. So far the discussion has been limited to factors affecting the apparent mixing requirements of flour. Figure 8 pictures loaves of bread which have been baked from water-extracted flour (the same flour as used for the curves of figures 5, 6, and 7) alone and in combination with the whole extract or gliadin.

Loaves A and B baked from the water-extracted flour, using a straight-dough procedure, exhibit "overoxidized" characteristics. The oxidation resulting from the long mixing time (24 minutes) is probably responsible for much of this effect. Limiting the fermentation time to 1 or 2 hours improved the characteristics.

A 100% sponge-dough procedure (C and D) improved the baking characteristics over the straight-dough procedure. (Freilich and Frey (6) showed that the characteristics of overoxidation were eliminated by the remix in a sponge dough.) This flour was extracted in a 1:10 sus-

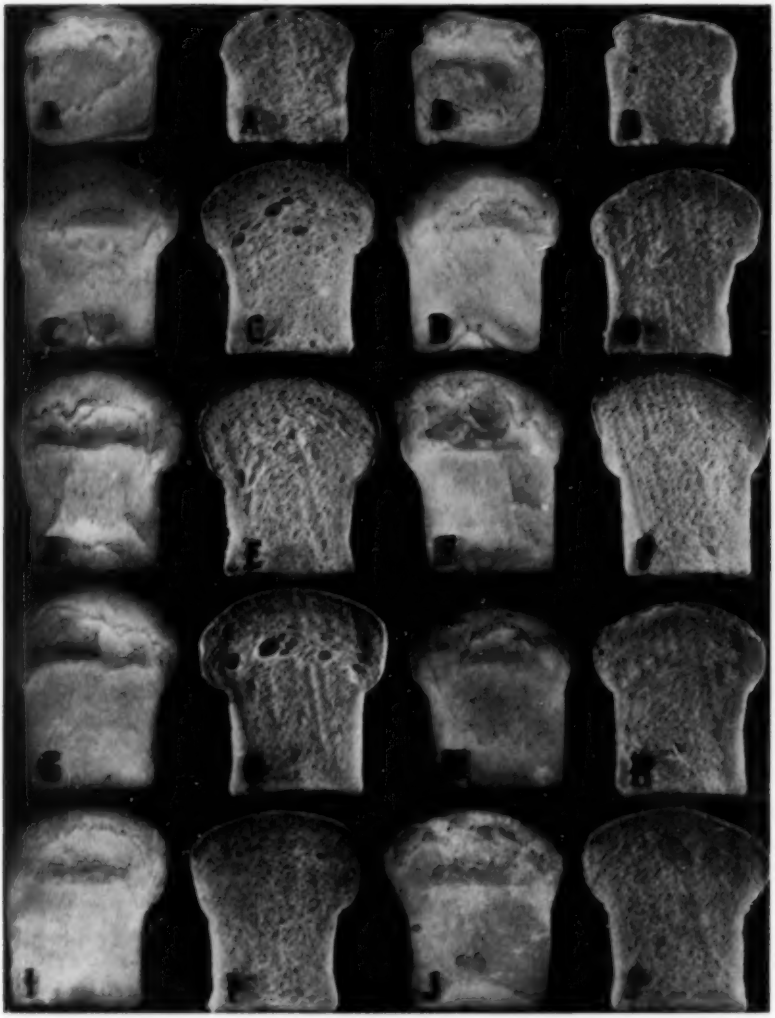

Fig. 8. Bread baked from a water-extracted commercial bakery flour (No. 4). A, water-extracted flour, using a straight-dough procedure (24 minutes mix); B, same as A with 1 mg.% potassium bromate; C, water-extracted flour using a 100% sponge-dough procedure (9 minutes remix); D, same as C with 1 mg.% potassium bromate; E, water-extracted flour, with total solubles using a 100% sponge-dough procedure (3½ minutes remix); F, same as E with 1 mg.% potassium bromate; G, water-extracted flour with 4% gliadin using a 100% sponge-dough procedure (4 minutes remix); H, same as G with 1 mg.% potassium bromate; I, non-treated commercial bakery flour using a 100% sponge-dough procedure (5 minutes mix); J, same as I with 1 mg.% potassium bromate.

pension (by weight); thus the solubles were removed before a gluten complex was formed. However, a fair baking product still remains if the dough is given an adequate remix.

A series of 100% sponge doughs (duplicates of dough for loaf C) remixed 6, 7, 8, 9, 10, and 15 minutes showed that the best bread quality was obtained at the peak on the remixing curve, and that the optimum mix as judged visually was giving good baking properties.

Total reconstitution was given in loaves E and F, and the loaves produced were excellent. These pictures point out the importance of the water-solubles of flour to the baking properties as well as the mixing requirements. Since many papers in the literature (using the reconstitution technique of combining starch and gluten) omit any reference to the mixing requirement, many of the differences attributed to solubles could be due to nonoptimum mixing.

The water-extracted flour was combined with 4% gliadin to produce loaves G and H. As usual the mixing requirement was short and the loaf properties good, but gliadin was not as effective as the whole extract. Gliadin prepared by the usual procedures is more likely to be denatured during preparation than is the gliadin in a water extract. Also other constituents in the water extract such as albumin and pentosan are reported to influence the baking properties (13, 14, 15). Therefore, differences between results with gliadin and with the water-solubles are to be expected.

Discussion

Extracting the water-solubles by dispersing flour in relatively large amounts of water (1:10 suspension) and allowing the insoluble portion to settle naturally prevents gluten formation and accordingly precludes the binding of the soluble proteins into a gluten complex. In this work 9.9%* [2] solubles were extracted using 1:10 flour suspensions of a spring-wheat blend. With winter wheat a range of 8.6% to 10.0%* solubles was obtained. Prentice *et al.* (18) recovered 5.9%* and 6.3%* solubles from the gluten washwater for two spring wheats. Finney (5) recovered 3.6%* solubles from the gluten washwater from Kharkof, a winter wheat. These data emphasize that widely varying amounts of solubles will be complexed in washed glutens, depending on the washing techniques. Washing doughs with distilled water removes more soluble protein from the gluten than does washwater containing various concentrations of salts. Inasmuch as the mixing requirement is influenced by the amount of the water-solubles remaining in the washed gluten,

[2] The asterisk indicates calculation on a dry-weight basis.

the mixing time could account for some of the discrepancies reported by various laboratories on the importance of the water-solubles of flour on the baking characteristics.

Gluten washed from a water-extracted flour is exceedingly short and consequently has poor gas-holding capacity, which again emphasizes the necessity of solubles for normal gluten properties. This was observed by Baker, Parker, and Mize (1) when solubles were removed from a gluten by successive suspensions with a Waring Blendor.

Removal of the water-soluble constituents of wheat flour extends the mixing time. Apparently the most effective water-soluble component for shortening mix requirements is gliadin. Flour extracted with sodium chloride solution (dialyzed and dried) retains its normal mix time. Since gliadin would not be removed under these conditions, it is to be expected that the mixing time would remain the same if gliadin is an important factor in determining the mixing requirement.

"Gliadin" prepared from the 70% alcohol (by weight) extract of a water-extracted flour was not quite as effective in controlling the mixing requirements as gliadin prepared by the normal procedures from washed gluten. This may indicate that the material of lower molecular weight is the more effective. Further support to this observation is given by comparison of the quantity of water-soluble protein extracted from short- and long-mixing varieties.

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A STUDY OF PROTEIN COMPOSITION IN BREAD DOUGH. I. CALCULATION OF AMOUNT OF NONFAT DRY MILK IN BREAD BY MEANS OF FORMOL-TITER DETERMINATIONS[1]

Helge Shipstead[2]

ABSTRACT

A modified formol titration method was developed for testing the proteins of wheat flours, nonfat dry milk (NFDM), and yeast. The formol titrations gave optimum values at pH end point of 9.00 and at a temperature of 25°C. Formol-titer values are calculated by dividing the formol nitrogen by the total nitrogen. To estimate the approximate amount of NFDM in bread, or its equivalent in regard to lysine, a chart was prepared based on determinations of total nitrogen calculated as percentage of moisture-free bread solids and the formol-titer of the bread proteins.

This study was undertaken to determine if the formol titration of the various proteins used in bread could be used to estimate the amount of milk proteins added to bread. Schiff (7) first observed that formaldehyde developed a certain amount of acidity when added to alkaline amino acid or protein solutions, and Steinegger (8) used

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this reaction to estimate the protein content of milk. Pyne (6) increased the accuracy of the formol titration of milk by adding potassium oxalate to precipitate the calcium. Linderstrøm-Lang (5) expressed the formol titration of casein as a fraction by dividing the percentage of formol nitrogen by the percentage of total nitrogen (Kjeldahl). The author proposes to call this fraction the "formol-titer."

The numerical value of the formol-titer of a protein complex or fraction will depend mainly on the total ϵ-amino groups available from lysine in all of the protein molecules present. Relatively small additions of free amino acids to a protein sample will increase the average formol-titer value, because free amino acids have very high formol-titers compared to proteins. According to Kekwick and Cannan (3), the end α-amino groups in proteins contribute only slightly to the formol titration of a protein when the end point of the titration is as high as pH 9. Since milk proteins have a much higher lysine content than wheat proteins, it should be possible to use the formol-titer as a means of estimating the amount of milk proteins in bread.

Development of Modified Formol Titration Procedure

Reagents: (1) A 10% solution of sodium salicylate solution for complete peptizing of proteins. (2) 2-Octanol to reduce foaming. (3) Sodium hydroxide, 1.0*N*, to improve dispersion. (4) Sodium hydroxide, 0.05*N*, for titration. (5) Formaldehyde solution, 36–38%, reagent quality.

Apparatus: (1) Waring Blendor with Variac transformer. (2) Beckman pH meter, battery-operated Model N. (3) Small glass stirrer.

Procedure: Of the sample to be tested use an amount that will give 5 to 10 ml. 0.05*N* sodium hydroxide solution for the formol titration. This amount depends on the protein content and its formol-titer. Weigh the sample to an accuracy of 0.01 g. and place it on top of 250 ml. 10% sodium salicylate solution in a Waring Blendor with 5 drops 2-octanol and enough normal sodium hydroxide solution to bring the pH to 8–9. Regulate the blender speed with the transformer at 70 volts; this will peptize the flour proteins in 10 minutes. Yeast samples require a higher blender speed at 90 volts for 30 minutes to break up the cell structure. Transfer the solution to a 600-ml. beaker and rinse the blender jar with about 50 ml. distilled water in three portions. Cool to 25°C. and adjust the pH to 9.00 by adding 0.05*N* sodium hydroxide from a microburet (10 ml.). Measure the

pH with a Beckman Model N pH meter, using a glass stirrer for quick distribution of the caustic. Check the correct adjustment of the pH meter frequently during the titration. Cover the beaker with a cardboard with suitable slots and holes for the electrodes, stirrer, and buret tips to prevent absorption of carbon dioxide from the atmosphere, which would tend to lower the pH. Allow 5 minutes to adjust the pH at exactly 9.00 by adding more caustic if required. At the end of the 5-minute period add 10.00 ml. nonneutralized concentrated formaldehyde solution and titrate immediately with 0.05*N* sodium hydroxide solution to the end point of 9.00 pH. There is usually a slight drop in pH with time, so allow another 5-minute period to adjust the pH at exactly 9.00 by adding more caustic. Deduct from this titration value a blank titration value obtained by using the procedure described above, but omitting the test sample. Check the blank titration value at 2-week intervals and especially when a new formaldehyde reagent is started.

TABLE I

EFFECT OF pH AT END POINT ON FORMOL TITRATION VALUES USING 10 ML. 36% FORMALDEHYDE SOLUTION PER TEST

PRODUCT	pH AT END POINT	0.05 *N* SODIUM HYDROXIDE USED IN TITRATION		
		Test	Blank	Net
		ml.	*ml.*	*ml.*
10.00 g. Flour	8.50	5.25	.93	4.32
	9.00	5.78	1.15	4.63
	9.50	6.46	2.01	4.45
2.00 g. NFDM	8.50	9.24	.93	8.31
	9.00	9.80	1.15	8.65
	9.50	10.29	2.01	8.28

TABLE II

EFFECT OF TEMPERATURE ON FORMOL TITRATION VALUES AT pH 9.00 END POINT

PRODUCT	TEMPERATURE OF TITRATION	0.05 *N* SODIUM HYDROXIDE USED IN TITRATION		
		Test	Blank	Net
	°C.	*ml.*	*ml.*	*ml.*
10.00 g. Flour	20	5.66	1.11	4.55
	25	5.94	1.15	4.79
	30	5.60	1.31	4.29
2.00 g. NFDM	20	9.67	1.11	8.56
	25	9.80	1.15	8.65
	30	9.59	1.31	8.28

Discussion of the Modified Formol Titration Method. In Table I are presented the results of experimental formol titrations with flour

and nonfat dry milk (NFDM) samples, including blanks made at three different pH end points. The net titration values of both samples (after deducting the blank values) show an optimum at pH 9. Formol titrations with both samples at a pH end point of 9.00 were carried out at three different temperatures: 20°, 25°, and 30°C. The results given in Table II show optimum net values at 25°C. When the amount of concentrated formaldehyde was increased from the customary 10 ml. per test to 20 ml., slightly higher net titration values were obtained with both samples. The blank value, however, was twice as high, and it therefore seemed advisable to use only 10 ml. formaldehyde solution per test. The formaldehyde solution was purposely made acid by the manufacturer at about pH 4 by adding 0.02 to 0.03% formic acid to prevent polymerization. It is therefore advisable not to neutralize it as often recommended in the past. The blank titration value is caused partly by the formic acid and partly by the acidic property of formaldehyde itself at pH 9, according to Levy (4). Sodium salicylate is used to peptize the wheat proteins completely and the blender action helps to break up any large protein particles. When titrating milk, the blender is not required if the milk proteins are soluble. The sodium salicylate changes the calcium phosphate caseinate complex into sodium caseinate, as judged by the complete disappearance of milkiness. This accomplishes the same effects as obtained by adding potassium oxalate to milk, namely a higher formol titration value and a more stable end point. The pH meter should preferably be a battery-operated instrument to ensure steady readings. The advantages of using a pH meter instead of the customary indicator phenolphthalein are twofold. First, the end point can be chosen to give optimum values, and secondly, the exact end point can be determined far more accurately with a pH meter than with an indicator.

The total nitrogen content of the test sample is determined by the Kjeldahl method and calculated as percent nitrogen on a standard moisture basis. The formol nitrogen content of the test samples is determined as described above and calculated as percent formol nitrogen on a standard moisture basis. The formol nitrogen of the test sample is the nitrogen of amino groups available for the reaction with formaldehyde and is equivalent to the net titration value of 0.05*N* sodium hydroxide (1 ml. 0.05*N* sodium hydroxide is equivalent to $0.014 \times 0.05 = 0.00070$ g. nitrogen).

The formol-titer of the test sample is calculated by dividing the formol nitrogen by the total nitrogen. The formol-titer is a value related to the type of proteins present in the test sample and is independent of the moisture content of the sample.

Formol-Titers of Various Bread Ingredients

Materials and Methods. Six different commercial wheat-flour samples with decreasing protein content, recommended for bread baking, were selected for testing. The wheat types, grades, percent flour extraction, and ash contents are given in Table III. All of the five experimental samples of NFDM had been heat-treated at 190°F. for 10 minutes to ensure good baking quality. The yeast samples, obtained from a local grocery store, were of two different brands.

TABLE III

WHEAT FLOUR SAMPLES USED IN FORMOL TITRATION TESTS

Flour Sample No.	Wheat Type	Grade	Flour Extraction	Ash on 14% Moisture Basis
			%	%
1	HRS	Long patent	98	0.47
2	HRS	Short patent	80	0.39
3	½ HRS + ½ HRW	Short patent	82	0.40
4	⅓ HRS + ⅔ HRW	Short patent	80	0.38
5	⅓ HRW + ⅔ Baart	Medium patent	85	0.39
6	HRW	Short patent	80	0.38

Three loaves of bread were baked, using flour No. 5, 2% yeast No. 2, 4% sugar, 2% shortening, and 2% salt. Bread No. 1 contained no milk, bread No. 2 had 6%, and No. 3 had 12% of NFDM sample No. 1. All percentages were based on flour with 14% moisture. After 2 days of storage the three loaves were prepared for analysis by cutting off all crust and then cutting the bread into small cubes, which were spread out to dry overnight. The dry crumbs were then pulverized, and each batch was placed in a tight container.

All of the various products described above were tested for moisture, total nitrogen, and formol nitrogen. The total and formol nitrogen values, calculated as percentage based on a standard moisture content, are given in Table IV together with the calculated formol-titers.

Results and Discussion. The formol-titers of the six samples of wheat flours tested had a tendency to increase with decreasing total nitrogen content. This is caused by variations in the various wheat protein fractions with their different lysine contents, and corresponding differences in formol-titers. The NFDM samples varied in formol-titers only from 0.0473 to 0.0480, although the total nitrogen content showed a variation of 5.80 to 6.13%. The two yeast cakes tested gave very high formol-titers with a relatively small difference. In Table V are given the calculated values of total and formol nitrogen in the three test breads. It is assumed that the sugar, fat, and salt used in

TABLE IV

FORMOL-TITERS OF VARIOUS BREAD INGREDIENTS

PRODUCT	SAMPLE NO.	STANDARD MOISTURE	TOTAL NITROGEN	FORMOL NITROGEN	FORMOL-TITER
		%	%	%	
Wheat flour[a]	1	14.0	2.46	0.0331	0.0134
	2	14.0	2.29	0.0318	0.0139
	3	14.0	2.05	0.0278	0.0135
	4	14.0	1.94	0.0296	0.0153
	5	14.0	1.89	0.0276	0.0146
	6	14.0	1.83	0.0271	0.0148
Av.		14.0	2.08	0.0295	0.0143
NFDM	1	3.50	5.80	0.279	0.0480
	2	3.50	5.81	0.277	0.0476
	3	3.50	5.88	0.278	0.0473
	4	3.50	6.13	0.295	0.0480
	5	3.50	6.12	0.293	0.0479
Av.		3.50	5.95	0.285	0.0478
Yeast cake	1	70.0	1.94	0.161	0.0830
	2	70.0	2.01	0.166	0.0824
Av.		70.0	1.98	0.164	0.0827
Bread crumbs	1[b]	6	2.07	0.0332	0.0160
	2[c]	0	2.32	0.0489	0.0211
	3[d]	0	2.52	0.0622	0.0247

[a] Wheat flour samples given in Table III.
[b] Wheat flour sample No. 5; no addition of NFDM.
[c] Wheat flour sample No. 5; 6% NFDM No. 1.
[d] Wheat flour sample No. 5; 12% NFDM No. 1.

TABLE V

CALCULATED VALUES OF TOTAL AND FORMOL NITROGEN IN TEST BREADS
(per 100 g. flour, 14% moisture)

	FLOUR NO. 5 14% M.B. F-T[a] 0.0146	NFDM No. 1 3.5% M.B. F-T 0.0480	YEAST NO. 2 70% M.B. F-T 0.0824	TOTAL	F-T CALCULATED VALUES	F-T TESTED VALUES[c]
	g.	g.	g.			
			Bread No. 1		0.0161	0.0160
Ingredient	100.0	0.0	2.03			
Total N	1.89	0.0	0.041	1.93		
Formol N	0.0276[b]	0.0	0.0034	0.0310		
			Bread No. 2		0.0209	0.0211
Ingredient	100.0	5.96	2.03			
Total N	1.89	0.346	0.041	2.28		
Formol N	0.0276[b]	0.0166	0.0034	0.0476		
			Bread No. 3		0.0245	0.0247
Ingredient	100.0	11.92	2.03			
Total N	1.89	0.692	0.041	2.62		
Formol N	0.0276[b]	0.0332	0.0034	0.0642		

[a] F-T = formol-titer.
[b] Formol nitrogen = total N × formol-titer ($1.89 \times 0.0146 = 0.0276$).
[c] See Table IV, last column, under "Bread crumbs."

the breads contain a negligible amount of nitrogen. The fractional values of total and formol nitrogen in the flour, NFDM, and yeast are calculated from values given in Table IV. These are added up

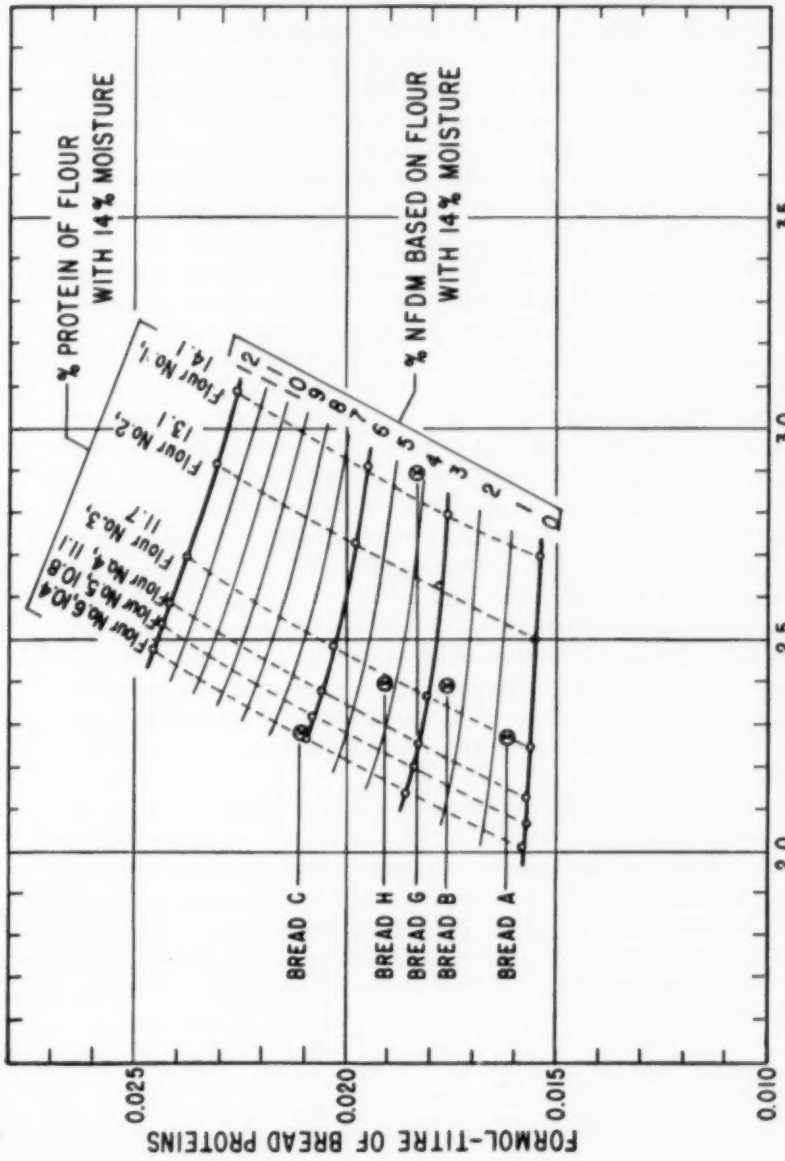
% PROTEIN OF FLOUR WITH 14% MOISTURE
% NFDM BASED ON FLOUR WITH 14% MOISTURE
Flour No. 1, 14.1
Flour No. 2, 13.1
Flour No. 3, 11.7
Flour No. 4, 11.1
Flour No. 5, 10.8
Flour No. 6, 10.4
0 1 2 3 4 5 6 7 8 9 10 11 12
BREAD A
BREAD B
BREAD G
BREAD H
BREAD C
2.0 2.5 3.0 3.5
TOTAL NITROGEN OF DRY BREAD SOLIDS, %
0.025 0.020 0.015 0.010
FORMOL-TITRE OF BREAD PROTEINS

Fig. 1. Chart for estimating the amount of NFDM in bread or its equivalent in regard to lysine.

for each bread, and the theoretical formol-titer is calculated. These values agree very well with actual test values given in Table IV for the three samples of bread crumbs.

Assuming that no nitrogen is lost during fermentation and baking, it was possible to calculate the loss of total bread solids. With no addition of NFDM this amounted to 1.6 g., and with the 6 and 12% addition of NFDM the loss was 2.1 g. per 100 g. of flour (14% moisture). This loss of solids had been caused by fermentation of the sugar and starch in the dough. With this information it was possible to calculate the percentage of total and formol nitrogen of moisture-free bread solids in breads made with the six samples of flour (given in Table IV). The formol-titers of these flour samples were chosen as the average of all samples at 0.0143. The addition of yeast, sugar, fat, and salt was assumed to be the same as used in the experimental breads. Additions of NFDM were calculated at three levels — 3, 6, and 12% — on the basis of flour with 14% moisture. The average values for NFDM were 5.95% N (based on 3.5% moisture) and a formol-titer of 0.0478. From the calculated values of total and formol nitrogen, based on 100 g. flour (with 14% moisture) with increasing protein content at three levels of NFDM, were calculated the formol-titers and the corresponding values of total nitrogen of dry bread solids. A chart (Fig. 1) was prepared by plotting formol-titers against percent total nitrogen in dry bread solids. Slightly curved lines (broken) were drawn through points for each flour at 0, 3, 6, and 12% NFDM. Slightly curved lines (solid) were also drawn through points for each level of NFDM with the six different flours. Lines for each percent NFDM from 1 to 11 were drawn in between in best proportions. In estimating the addition of NFDM to bread, the maximum error caused by variations in the formol-titer of the flour from the average value of 0.0143 is about ± 1%.

The formol-titers and total nitrogen on a dry basis were calculated for three breads made with flour No. 3 and containing no NFDM, but including instead the following components: (A) 1% more yeast than the usual 2%; (B) 3% soya flour with 8% moisture; 6.02% N and a formol-titer of 0.04; and (C) 0.083% lysine (equivalent to 3% NFDM) with 19.2% N and a formol-titer of 0.73. The calculated values were located on the chart, and the corresponding amounts of equivalent additions of NFDM were estimated as follows:

	NFDM equivalent
	%
1% extra yeast	0.7 NFDM
3% soya flour	2.5 NFDM
0.083% lysine	6.1 NFDM

There is a possibility that synthetic L-lysine, with or without other essential amino acids, may someday be added to bread to improve its nutritional value, according to Flodin (2). The high formol-titer of 0.73 for lysine indicates that the α-amino group of a "free" amino acid contributes to a considerable degree to the formol titration. This is in contrast to the α-end amino groups in proteins (3). According to the chart, the estimated amount of NFDM equivalent to 0.083% lysine is therefore much too high. Such addition of lysine or other "free" amino acids to bread must be declared on the label to conform with the present federal and state standards of identity.

The effect of additions of fungal proteases to dough on the formol-titer of the bread might be very interesting to investigate. It is rather unlikely, however, that the minute additions recommended for improvement of certain types of flour will cause any marked increase in "free" amino acids with correspondingly higher formol-titers.

Larger additions of sugar, fat, or salt to the dough than those assumed in the calculations of the chart in Fig. 1 will result in a lower percentage of nitrogen in the moisture-free bread solids. This will tend to make the estimation of added NFDM too low, whereas smaller additions of sugar, fat, or salt would increase the NFDM estimate.

Two commercial loaves of white wheat bread (samples G and H) were obtained from a local grocery store. According to the labels nothing unusual had been added to either of them, although sample G contained honey and butter. The two loaves were prepared as described above, and the crust-free dry bread crumbs were analyzed for moisture, total nitrogen expressed as percentage on a moisture-free bread solids basis, and formol-titer.

Sample G

Moisture content of bread crumbs, 9.12%
Total nitrogen on moisture-free basis, 2.90%
Formol-titer of bread proteins, 0.0184

These values were located on the chart at point G, which indicates an addition of 4.2% NFDM or its equivalent in regard to lysine. The position of point G on the chart also indicates that a flour with a relatively high protein content had been used. The label actually states that the bread was made with "unbleached hi-protein flour."

Sample H

Moisture content of bread crumbs, 8.54%
Total nitrogen on moisture-free basis, 2.40%
Formol-titer of bread proteins, 0.0191

These values are located at point H on the chart, which indicates an

addition of 4.2% NFDM or its equivalent in regard to lysine. It also indicates that the flour used had about 11.6% protein on 14% moisture basis.

Choi *et al.* (1) have developed another method for estimating the amount of NFDM added to bread. This method is based on the determination of lactose after fermenting out all other reducing sugars. This method, however, would give misleading results if whey powder with high lactose or a special high-protein dry milk with low lactose content had been used in the dough instead of normal NFDM. By combining the two methods it would be possible to estimate fairly closely the actual addition of NFDM in bread.

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COOKING QUALITY OF 26 VARIETIES OF MILLED WHITE RICE[1]

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ABSTRACT

The cooking qualities of 26 varieties or selections of milled white rice were evaluated by some objective measurements of physical and chemical characteristics of the rice and by palatability tests. Variety and environment as well as grain type influenced cooking quality. Long-grain varieties of rice tended to absorb more water than the other grain types, although there were exceptions. Variations in starch and total solids in the residual cooking liquids were apparently due to varietal factors, although the production source of the variety may have been an influencing factor. Highly significant correlations were found among some of the objective measurements and palatability evaluations. With a larger number of varieties, the possibility of developing equations for predicting some palatability characteristics of rice from the objective measurements seems promising.

In the past, the selection of varieties of rice for commercial production in the United States has been based on such factors as range of adaptation to climatic conditions, disease resistance, yield, and suitability for mechanical harvesting and artificial drying. Evaluation techniques have been established for these factors, but there is little information available in the literature on methods for determining or predicting the cooking and eating qualities of rice varieties. It is now recognized that the plant breeder needs tests for all of these factors to guide him in his efforts to produce new strains and varieties of rice, not only with acceptable field and harvesting characteristics but with cooking and processing behavior that meets specific consumer requirements. Consumers vary greatly in their preference for cooked rice. Some prefer that it be a fluffy, dry, or flaky product; others desire a moist, sticky rice. The rice industry must supply rices that meet the requirements of both groups. There are also the commercial processors who have their own requirements for rice to be canned, made into dry quick-cooking rice, parboiled, or used in soups or other formulations. But whether rice is consumed in the home or preprocessed in the factory, its behavior toward hot or boiling water is the property that must be understood, and it was the purpose of this investigation to learn how rices differ from each other in this respect.

Test methods for differentiating the cooking qualities of rice developed in this laboratory were applied to eight varieties of rice in a previous study (1).

[1] Manuscript received November 30, 1956.

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In order to further evaluate these test methods and to determine the cooking quality of a larger number of varieties of rice, the present study was undertaken on 26 varieties of milled white rice grown in different locations in different crop years.

Materials and Methods

Included in the study of the evaluation of cooking quality were 66 sample lots of rice representing 26 varieties or strains and crops from four states which were grown in 1953, 1954, or 1955; 56 lots were samples from the Beaumont, Texas, Field Plot Variety Tests. Since the samples from Beaumont were grown under the same conditions, differences in cooking quality among these lots could be attributed to varietal factors. Unfortunately, palatability tests could not be made on some of the lots grown in 1954, because of the limited quantities of these samples. Evaluations for differences due to environment and age as well as to variety were made on the remaining lots of rice. All tests were made during the spring of 1956.

The cooking qualities of the various rices were evaluated by physical, chemical, and sensory panel methods. The tests included measurements of palatability, water absorption, and volume of cooked rice; and estimated starch, total solids, and pH of the residual cooking liquid. The equipment and procedures used were described in a previous paper (1); a few changes were made in the procedure. All sample lots of rice were cooked 20 minutes. The residual cooking liquids were cooled for about 3 hours in a constant-temperature room (24°C.) before the determinations of starch were made. When temperature was controlled, less variation among replications was observed. The intensity of the blue starch iodine color was determined by measurement of percent transmission at 600 mμ, the wave length of maximum absorbance of dilute amylose-iodine solution. A Bausch & Lomb Spectronic 20 colorimeter was used for the transmission measurements. The readings were made on solutions containing 0.5 ml. residual cooking liquid, distilled water to make 100 ml., and 2 ml. iodine solution (2 g. iodine + 20 g. potassium iodide in 1 liter of water). Since in this study only one cooking time was used and the total volume of liquid was similar in all cases, it was not necessary to convert the percent transmission readings into estimated starch contents as in the previous study. High transmission readings indicate low starch contents.

For the palatability tests, 100-g. samples of rice were cooked with boiling distilled water in baking dishes in a 350°F. oven, covered for a designated period of time and then uncovered for an additional 5 minutes. The results of preliminary tests were used to determine the

amount of water and the cooking time for each variety of rice grown in 1953 and 1954, on which palatability tests were made. All lots of one variety in this phase of the study were cooked in the same amount of water for the same length of time. However, the results indicated a possibility of overcooking or undercooking for different lots of the same variety.

Some lots of a variety required more water than others. When too little water was used, the cooked rice was apt to be hard and brittle and to have small volumes. With too much water, the rice was apt to be very pasty, with the grains set in a sticky mass formed by the starch of the rice grains and the excess water. Therefore, for the 1955 crops, the water-uptake ratios were used as a basis for determining the

SCORE CARD FOR EVALUATING COOKED RICE

Judge's Name _____ Date _____

CODE NO.				
SAMPLE	A	B	C	D
Color: 9 White 7 Cream 5 Yellow 3 Tan 1 Brown				
Cohesiveness: 9 Well-separated grains 7 Partially separated 5 Sticky 3 Very sticky 1 Pasty				
Off-flavor: 9 None 7 Perceptible 5 Slightly strong 3 Moderately strong 1 Very strong				
Doneness: 9 Underdone 7 Slightly underdone 5 Done 3 Slightly overdone 1 Overdone				

Comments:

_____

amount of water to be used for cooking each lot of rice, as follows: For a lot of rice with a water-uptake ratio of 3.15, 250 ml. of water were used; for each 0.10 change in the water-uptake ratio, the volume of water was altered 10 ml. All samples for palatability tests from the 1955 crop were cooked for 28 minutes.

In evaluating the samples in the palatability tests, a trained tasting

TABLE I

COOKING CHARACTERISTICS OF DIFFERENT VARIETIES OF RICE

GRAIN TYPE AND VARIETY	LOT NO.	SOURCE	CROP YEAR[a]	WATER-UPTAKE RATIO	VOLUME OF COOKED RICE FROM 8 G. UNCOOKED RICE	RESIDUAL COOKING LIQUID		
						Transmission	Total Solids	pH
					ml.	%	%	
LONG-GRAIN								
Bluebonnet	1	Tex.	1954	3.32	46	46	9.4	6.36
Bluebonnet 50	2	Tex.	1954	3.26	43	48	9.7	6.43
	3	Tex.	1954	3.26	45	46	9.7	6.59
	4	Tex.	1954	3.21	45	47	10.8	6.87
	5	Tex.	1955	3.23	44	43	10.2	6.46
Century Patna 231	6	Tex.	1953	3.48	49	72	9.1	6.44
	7	Tex.	1953	3.50	50	72	8.4	6.44
	8	Tex.	1954	3.38	47	64	10.0	6.42
	9	Tex.	1954	3.30	45	57	11.3	6.56
	10	Tex.	1954	3.45	47	70	9.2	6.73
	11	Tex.	1955	3.20	46	62	10.8	6.52
Fortuna	12	Tex.	1954	3.12	42	48	10.3	6.46
	13	Tex.	1955	3.03	42	45	11.2	6.61
Improved Bluebonnet	14	Tex.	1954	3.24	44	40	10.4	6.35
	15	Tex.	1955	3.24	45	40	10.0	6.44
Rexark	16	Tex.	1954	3.32	45	63	9.4	6.62
Rexoro	17	Tex.	1953	3.48	49	46	9.1	6.38
	18	Tex.	1954	3.45	47	45	10.6	6.94
	19	Tex.	1954	3.31	47	39	11.3	6.56
	20	Tex.	1955	3.31	46	38	10.4	6.46
Sunbonnet	21	La.	1954	3.41	44	45	10.8	6.62
	22	Tex.	1954	3.27	43	40	11.6	6.54
	23	Tex.	1955	3.19	43	41	11.1	6.42
Texas Patna	24	Tex.	1954	3.44	46	40	11.8	7.78
	25	Tex.	1954	3.32	48	37	10.4	6.30
	26	Tex.	1955	3.30	46	39	10.8	6.47
Toro	27	La.	1954	3.40	47	64	11.7	6.72
	28	La.	1954	3.43	47	66	10.8	6.67
	29	Tex.	1955	3.33	46	60	11.1	6.56
TP 49	30	Tex.	1954	3.19	44	40	10.7	6.42
	31	Tex.	1955	3.26	44	39	11.4	6.56
B4512A1-20	32	Tex.	1954	3.54	48	40	9.9	6.50
B4512A1-32	33	Tex.	1955	3.36	46	44	9.5	6.51
B455A1-25	34	Tex.	1954	3.27	47	38	10.7	6.40
	35	Tex.	1955	3.25	44	41	10.4	6.59
SP-6	36	Ark.	1953	3.27	46	60	10.4	6.96

TABLE I (Continued)

Grain Type and Variety	Lot No.	Source	Crop Year[a]	Water-Uptake Ratio	Volume of Cooked Rice from 8 g. Uncooked Rice	Residual Cooking Liquid		
						Transmission	Total Solids	pH
					ml.	%	%	
Medium-Grain								
Blue Rose	37	Tex.	1954	2.84	40	64	10.1	6.56
	38	Tex.	1955	2.84	39	60	10.1	6.63
Calrose	39	Tex.	1954	3.14	41	64	10.4	6.66
	40	Calif.	1954	2.96	41	47	14.5	6.90
	41	Tex.	1955	3.01	40	63	9.7	6.64
Early Prolific	42	Tex.	1954	2.98	42	68	8.7	6.52
	43	Tex.	1955	3.07	42	67	9.0	6.61
Magnolia	44	Tex.	1954	3.02	40	79	6.7	6.54
	45	Tex.	1955	2.94	41	78	6.9	6.64
Nato	46	Tex.	1954	3.12	42	70	8.1	6.55
	47	Tex.	1955	3.11	43	69	8.9	6.57
Zenith	48	Tex.	1953	3.27	46	72	8.2	6.61
	49	Tex.	1953	3.36	46	67	9.0	6.67
	50	Tex.	1954	3.17	44	58	9.9	6.56
	51	Ark.	1954	3.17	45	59	11.3	6.90
	52	Tex.	1955	3.18	44	62	9.3	6.61
Short-Grain								
California Pearl	53	Calif.	1954	3.03	41	47	13.3	6.79
Caloro	54	Tex.	1953	3.34	45	64	10.2	6.59
	55	Tex.	1953	3.29	46	61	9.3	6.62
	56	Tex.	1954	3.14	42	54	10.9	6.54
	57	Calif.	1954	2.87	40	45	14.3	6.90
	58	Calif.	1955	2.89	38	40	15.1	6.84
	59	Tex.	1955	3.02	42	58	10.4	6.55
Colusa 1600	60	Tex.	1954	2.96	40	56	10.1	6.67
	61	Calif.	1954	2.88	40	40	14.4	6.91
	62	Tex.	1955	3.02	40	52	11.0	6.72
Hyb Mix 11-47-11-1	63	Tex.	1954	3.26	44	42	10.8	6.61
	64	Tex.	1955	3.16	43	44	10.4	6.62
Hyb Mix 12-47-6-2	65	Tex.	1954	3.33	44	53	11.8	6.54
	66	Tex.	1955	3.13	42	56	11.4	6.57

[a] Tested in the spring of 1956.

panel of six members scored the samples for the palatability factors of color, cohesiveness, off-flavor, and degree of doneness, using the score card given herewith. In determining the order of testing the samples, randomized block designs were used and two to five replications were made of each test for each sample lot of rice.

Results and Discussion

Physical and Chemical Tests. The results of the physical and chemical tests are summarized in Table I.

Grain type appeared to be an influencing factor in water absorption of rice in that most of the long-grain varieties absorbed more water than either the medium- or short-grain types, although there was some overlapping of water-uptake ratios among the three grain types. Fortuna rice absorbed much less water than any of the other long-grain varieties grown during the same years. The short-grain "Hyb Mix" strains absorbed much more water than the other short-grain varieties and most of the medium-grain rices, and more than one long-grain rice.

As might be expected, samples that had high water-uptake ratios tended to yield large volumes of cooked rice. Volumes ranged from 38 to 50 ml. per 8 g. of raw rice.

It is reported in the literature that different varieties of rice have approximately the same amount of starch in the milled grain (3). When rice is cooked in excess water, the residual cooking liquids of some varieties contain more total solids and starch than do other varieties. In most of the residual cooking liquids, the total solids content expressed as percent of raw rice ranged between 9 and 11%. Exceptions were the residual liquids from Magnolia medium-grain rice which contained 7% total solids, and the liquids from the rices grown in California (Calrose, Caloro, and Colusa) which contained 13% or more.

In general, the residual cooking liquids from the long-grain rices had lower transmission readings (indicating high starch contents) than the liquids from the short- and medium-grain rices, although there were some exceptions. The residual cooking liquids from Century Patna 231, Rexark, SP-6, and Toro rices had higher transmission readings than the other long-grain rices. In a starch-iodine test on ground milled rice and water, Halick and Keneaster (2) also found Century Patna 231 to have a much higher percent transmission or iodine-blue value than Texas Patna, Rexoro, and Bluebonnet 50.

For the lots of rice tested, the average transmission reading for long-grain rice was 48%, for medium-grain 67%, and for short-grain 49%. Rices grown in California had residual liquids with lower transmission readings than the lots of the same varieties grown in Texas. Additional lots of rice may, therefore, alter these averages.

Most of the residual liquids had mean pH values between 6.30 and 6.96. The residual liquid from Texas Patna rice grown on sandy-loam soil in Texas had a mean pH of 7.78, the only alkaline liquid, while the liquid of the same variety grown the same year on Beaumont clay soil had the lowest pH, 6.30. The residual cooking liquids from the long-grain varieties tended to have lower pH values than the other two grain types grown under similar conditions.

Some differences among lots of rice grown in different locations were significant. The lots of rice grown in California absorbed less water and had lower volumes when cooked, and the residual cooking liquids contained more total solids, had lower transmission readings, and higher pH values than did comparable lots of the same varieties grown in Texas. However, the available samples were too few to permit definite conclusions as to the environmental influence on cooking quality.

Palatability Evaluations. The results of the palatability tests are given in Table II. In preparing rice for the palatability tests, long-grain varieties required more water than short- and medium-grain types; some varieties within a grain type required more water than others.

Variations in color, cohesiveness, and off-flavor were noted among different lots of the same variety of rice. The differences in color of the lots of rice could not be attributed entirely to age, environment, or any other known factor. The lots of rice grown in California tended to be more cohesive or sticky than those of the same varieties grown in Texas. In off-flavor, the age of the rice when tested was apparently the most important influencing factor. Many of the lots of rice grown in 1953 and tested in the spring of 1956 were rated as stale in flavor. The most off-flavor was noted in Zenith, lot 48, which was described as rancid and "cardboard." Other 1953 crops with very poor flavors were Caloro, lot 54, described as "starchy," "rancid," and "cardboard"; and SP-6, cited as being "musty."

Differences in color and cohesiveness among varieties were noted by the panel. Some of the differences could be attributed to grain type. The long-grain varieties tended to be more white and less creamy than either of the other grain types. The long-grain varieties also tended to be less cohesive or sticky (have higher mean scores), although there were exceptions. Magnolia, a medium-grain rice, was less sticky than Toro and B455A1-25, both long-grain varieties. Toro apparently cooks more rapidly than the other varieties, since every lot of this variety that was tested was considered to be overdone by the panel. Samples considered by the panel to be overdone were also thought to be more cohesive. Long-grain rice tended to be less sticky when cooked 28 minutes than when cooked for a longer time.

Results of statistical analysis of the 1955 samples showed that a highly significant positive correlation of 0.674 existed between the water-uptake ratio and cohesiveness scores, and a highly significant negative correlation of 0.558 between total solids in the residual cooking liquid and cohesiveness scores. Using water-uptake ratio, total

TABLE II

Mean Palatability Scores for Different Varieties of Rice

Grain Type and Variety	Lot No.	Source	Crop Year [a]	Volume Water Used with 100 g. Uncooked Rice	Cooking Time	Mean Palatability Scores [b]			
						Color	Cohesiveness	Off-Flavor	Doneness
				ml.	*min.*				
Long-Grain									
Bluebonnet 50	3	Tex.	1954	250	28	8.7	6.5	8.2	4.8
	4	Tex.	1954	250	28	8.5	6.8	8.3	5.0
	5	Tex.	1955	260	28	8.0	6.1	8.0	4.8
Century Patna 231	6	Tex.	1953	250	28	6.8	7.2	6.6	5.3
	7	Tex.	1953	250	28	7.8	6.5	7.2	3.8
	9	Tex.	1954	250	28	8.3	6.9	8.2	4.7
	10	Tex.	1954	250	28	8.3	6.8	8.0	4.7
	11	Tex.	1955	250	28	6.8	6.0	7.7	4.5
Fortuna	13	Tex.	1955	240	28	7.0	5.6	7.8	5.3
Improved Bluebonnet	15	Tex.	1955	260	28	8.0	6.6	8.4	5.0
Rexoro	17	Tex.	1953	250	28	8.3	7.8	8.2	5.3
	18	Tex.	1954	250	28	8.0	7.2	8.3	5.2
	20	Tex.	1955	270	28	7.9	6.6	8.1	5.4
Sunbonnet	21	La.	1954	250	28	6.3	4.8	6.7	4.8
	23	Tex.	1955	250	28	7.2	5.8	8.5	5.1
Texas Patna	24	Tex.	1954	250	26	7.5	7.2	8.3	4.3
	26	Tex.	1955	260	28	8.0	6.6	8.3	4.8
Toro	27	La.	1954	250	28	5.5	4.4	6.2	3.8
	28	La.	1954	250	28	6.7	5.5	7.5	4.0
	29	Tex.	1955	260	28	7.4	5.0	7.8	4.1
TP 49	31	Tex.	1955	260	28	7.7	6.2	8.0	4.9
B4512A1-32	33	Tex.	1955	270	28	8.4	6.5	7.9	5.0
B455A1-25	35	Tex.	1955	260	28	7.8	5.6	8.3	5.2
SP-6	36	Ark.	1953	250	28	6.8	6.1	5.2	4.8
Medium-Grain									
Blue Rose	38	Tex.	1955	220	28	7.3	4.5	7.0	5.2
Calrose	40	Calif.	1954	220	30	6.0	3.3	7.8	4.7
	41	Tex.	1955	240	28	6.5	4.8	7.7	4.8
Early Prolific	43	Tex.	1955	240	28	6.9	5.3	6.8	5.2
Magnolia	45	Tex.	1955	230	28	6.4	6.4	7.5	5.8
Nato	47	Tex.	1955	250	28	6.5	4.4	6.2	4.5
Zenith	48	Tex.	1953	220	26	4.7	6.6	4.4	5.7
	49	Tex.	1953	220	26	7.2	5.8	6.9	5.5
	51	Ark.	1954	220	26	5.5	5.2	6.8	5.2
	52	Tex.	1955	250	28	7.4	5.1	7.2	4.8
Short-Grain									
California Pearl	53	Calif.	1954	220	28	6.7	3.9	7.7	5.0
Caloro	54	Tex.	1953	220	26	5.5	5.3	5.8	5.5
	55	Tex.	1953	220	26	8.0	5.7	7.6	5.0
	57	Calif.	1954	220	26	6.3	4.8	7.4	4.8
	58	Calif.	1955	220	28	6.1	3.1	7.3	4.3
	59	Tex.	1955	240	28	6.8	4.9	7.4	4.5
Colusa	61	Calif.	1954	220	26	7.0	4.4	8.0	5.0
	62	Tex.	1955	240	28	7.5	4.1	8.0	4.2
Hyb Mix 11-47-11-1	64	Tex.	1955	250	28	6.8	5.2	7.9	5.3
Hyb Mix 12-47-6-2	66	Tex.	1955	250	28	7.0	4.3	7.6	4.3

[a] Tested in the spring of 1956.
[b] Mean of 12 to 30 scores (six panel members, two to five replicates); see Score Card for interpretation of scores.

solids, and cohesiveness scores in a three-way correlation, a highly significant correlation coefficient of 0.740 was obtained. Water-uptake ratio and flavor scores also were related, with a highly significant correlation coefficient of 0.675, indicating that the higher the water-uptake ratio the less off-flavor was observed. Correlations among other quality characteristics were not significant for the limited number of samples available. Examination of a larger variety of samples may reveal statistically significant correlations among the observed relationships between percent transmission and cohesiveness scores.

Data obtained in this study on the palatability, water absorption, volume of cooked rice, amylose starch, total solids, and pH of the residual cooking liquid for short-, medium-, and long-grain varieties of rice will be useful for comparison of new varieties whose cooking qualities are not yet known. As more data are accumulated, it may be possible to develop equations for predicting the palatability characteristics of rice from the objective measurements of some of the physical and chemical properties of rice grains.

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COMPOSITION OF THE PROTEIN OF WHOLE MAIZE SEEDS[1]

MIRIAM WOLFE and L. FOWDEN[2]

ABSTRACT

The complete amino acid composition of the protein of seven varieties of maize seed has been determined. In order to reduce analytical errors due to losses of amino acids occurring during protein hydrolysis by mineral acids, protein fractions, representative of the total seed protein complements, were isolated and purified before subsequent hydrolysis. Amino acids present in the hydrolysates were estimated by a quantitative paper chromatographic technique.

Considerable differences occurred in the amino acid composition of the protein present in different varieties of maize seed, the largest differences being found for arginine, histidine, lysine, leucine, threonine, valine, and amide-nitrogen. The differences of protein composition tended to be greatest between varieties genetically most distantly related. These differences were not due to variations in the proportions of zein in the various seed proteins.

Many workers have studied the composition of the protein of maize seeds. The purpose of these investigations has usually been to assess the nutritive value of a cereal protein in comparison with other protein of vegetable or animal origin. Thus there has been a tendency to determine only the ten "essential" amino acids; in fact many investigators have determined only a limited number of these, apparently chosen for the ease with which they can be determined by specific assay methods. Since such studies yield an incomplete picture of protein composition, their value in comparative biochemistry is limited.

Most analyses of cereal protein appearing in the literature are open to criticism of a more serious nature for the following reasons. The normal method of hydrolyzing protein using hot mineral acids results in partial destruction of certain amino acids, particularly the sulfur-containing, basic, and aromatic types, and a production of insoluble humin, especially if carbohydrate is present as an impurity in the protein. Appendices in Chibnall (1) draw attention to this point, and indicate that losses during hydrolysis of cyst(e)ine, methionine, and tyrosine may reach 30%, and those of arginine, histidine, and lysine may be appreciable even in a partially purified protein. Losses of these amino acids tended to be greater as the purity of the hydrolyzed protein sample decreased. Since in most of the recorded analyses of maize protein, the ground seeds (containing up to 90% carbohydrate and other impurities) were hydrolyzed directly without any prior attempt to purify the seed protein, the resulting losses of labile amino acids may have been very serious.

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A further objection to the use of ground whole seed lies in the fact that the protein of the meal is less readily dissolved than a purified protein, and so incomplete hydrolysis to amino acids may result and give rise to a further source of error.

Presumably these types of error are responsible for the fact that in investigations where complete analyses of maize protein have been made, the total of the individual amino acid contents usually has not accounted for all the protein-nitrogen. Sauberlich, Chang, and Salmon (14) could account for about 85% of the total seed-nitrogen in the form of amino acids after hydrolysis.

While individual proteins, such as zein, have been isolated from maize seeds and purified before hydrolysis and analysis, the authors are not aware of any work in which *all* the protein present in the seeds has been treated in this way. But unless this is done, the inevitable errors of the analytical determinations may be sufficiently large to invalidate any conclusions made regarding the composition of the complete protein of the seeds.

The first task of the present investigation was to obtain purified protein fractions from maize seeds which were representative of the whole seed protein (i.e., the isolated protein should represent virtually 100% of the seed protein) and which could be hydrolyzed without humin formation. When this was achieved, it was found that the amino acids produced by acid hydrolysis accounted for nearly 100% of the total protein-nitrogen. A comparison of the protein composition of different varieties of East African maize seed has been made. Previous work had indicated that both the amount of protein and its composition may vary in different varieties of maize; the differences in the amino acid composition of the protein have been ascribed largely to the fact that, as the total protein present in the seed increases, zein represents an increasing percentage of protein (8, 9, 10, 15, 16). Zein, when compared with most other plant proteins, is relatively deficient in the amino acids lysine, tryptophan, and valine, and so, in varieties having markedly different total protein contents, these amino acids should be present to different extents in the seed protein. Frey (8, 9), using numerous varieties having protein contents in the range of 4.9–18.4% of the air-dry seed weight, has confirmed that the tryptophan and valine contents of the whole seed protein decreased as the total protein content increased. Sauberlich *et al.* (14) included all the amino acids in their studies, and showed that when the protein content of maize seeds was altered, the arginine, glycine, tryptophan, lysine, and, to a lesser extent, valine and threonine contents of the seed protein decreased with increasing total protein content, whereas the amounts

of leucine, alanine, phenylalanine, and proline were increased. In contrast to these observations, Miller, Aurand, and Flach (12) could not detect any significant differences in the methionine, tryptophan, or lysine contents of whole seed protein of some ten samples of maize, with protein contents in the range of 8.9–13.0%. The seven varieties of maize seed we have studied had similar total protein contents (8.2–10.3% by weight of the air-dried seeds), and hence they presumably had similar zein contents. Differences in the amino acid composition of the seed protein of different varieties were still apparent, however, and these differences tended to be larger between varieties that were genetically more distantly related.

Materials and Methods

Origin of the Maize Seed Analyzed. All the seed was grown at the Cotton Research Station, Namulonge, Kampala, Uganda, East Africa, on the same soil type—a dark-brown clay loam of high fertility. It represented three main named varieties: K8, Ulu, and Muratha. K8 and Muratha varieties are fundamentally white-grained; Ulu is yellow-grained. However, since maize is naturally out-pollinated, off-type colors can occur. Thus red and yellow together with white grains can be obtained on the same cobs of the K8 variety by direct pollination of the mother cobs with foreign pollen. The K8 white, K8 red, and K8 yellow seed samples analyzed were obtained from cobs having grains of such mixed colors. The white- and yellow-grain types of the Muratha variety had a somewhat different origin; the yellow grains were the result of a partial segregation from an earlier outcross with a yellow-grained maize. The yellow and white Muratha seeds analyzed, therefore, came from different cobs. The two samples of Ulu seed were of dent and flint types respectively, and so differed in grain-shape and hardness characteristics, which are inherited independently of the color of the grains. While there is every gradation between flint and dent, the samples of Muratha seed used were largely dent, and the K8 seed was mixed.

Isolation and Purification of Seed Protein. All the seed was first ground to pass through a 40-mesh sieve. About 5 g. of each sample were then twice extracted for 24-hour periods with 25 ml. of 99% *iso*propyl alcohol to remove fatty substances. Protein was extracted from the dried residues by a slight modification of the method of Foster, Yang, and Yui (5). The residues were shaken for five 48-hour periods with 25 ml. of a pH 10–11 borate buffer (prepared by the addition of 0.2*N* sodium hydroxide to a M/5 boric acid solution containing M/5 potassium chloride to give pH 10, followed by dilution to

give a M/20 borate buffer), containing 1% Teepol 530 (supplied by Shell Chemicals Ltd.) and 0.2% sodium bisulfite. The five extracts were pooled, and measured amounts were taken for micro-Kjeldahl nitrogen estimations by the procedure of Chibnall, Rees, and Williams (2). Ninety-six to 98% of the total nitrogen present in the ground seeds was extracted by this procedure.

The pH's of the extracts were adjusted to that of the isoelectric point of most proteins (pH 4.0–4.5) by addition of acetic acid, and protein precipitation was completed by warming them to 50°–60°C. for 30 minutes. The protein fractions were collected by centrifugation and were washed repeatedly with alternating hot water and hot 5% trichloracetic acid solution. (Hot alcohol was not used because a portion of the protein, zein, was soluble in it.) Finally the protein fractions were washed with ether and dried at room temperature. These protein fractions contained 96% or more of the nitrogen originally extracted from the seed. They may then be considered to be representative of the total protein of the seed. Nonprotein nitrogen must then represent not more than 4% of the total seed nitrogen.

Protein Hydrolysis. The protein fractions were hydrolyzed by heating with a mixture of equal volumes of 10*N* hydrochloric acid and glacial acetic acid in sealed ampoules at 100°C. One milliliter of acid mixture was used for each 3 mg. of protein sample. Stannous chloride dihydrate was added to the mixture of acids at a concentration of 1%, since Kofranyi (11) and Fowden (7) found that this reducing agent eliminated or markedly diminished humin formation during the hydrolysis of protein samples. Hydrolysis was allowed to continue for 18 hours; this hydrolysis time produced the maximum amount of alpha-amino nitrogen from each protein sample. The hydrolysates were evaporated to dryness repeatedly *in vacuo* at 50°–60°C., and the final residues of amino acids were dissolved in water to give solutions containing 0.45–0.90 mg. alpha-amino nitrogen per ml.

Total Alpha-Amino Nitrogen Determination. These were made by the method of Yemm and Cocking (20), after any ammonia released during the protein hydrolysis had first been removed by treatment with N/10 sodium hydroxide solution (6).

Amino Acid Estimations. Amino acids present in the protein hydrolysates were estimated after their separation on two-dimensional filter-paper chromatograms. One-tenth-milliliter samples of the solutions containing 0.45–0.90 mg. alpha-amino nitrogen per ml. (see above) were used for each chromatogram. The solvents used to develop the chromatograms were those of Wolfe (19); a one-phase solvent obtained by mixing five volumes of *n*-butanol, three volumes of methyl-

ethyl ketone, and two volumes of 8.5*N* ammonia solution was used for development of the chromatograms in the first direction, and the organic layer formed by equilibration of a mixture of four volumes of *n*-butanol, five volumes of water, and one volume of glacial acetic acid was used as the second solvent. Whatman No. 3MM filter paper was used throughout. Standard chromatograms containing known amounts of each amino acid were developed (in triplicate) under the same conditions. In this way recovery values were obtained for each amino acid and so accurate correction factors could be applied to all amino acid determinations made on the protein hydrolysates. The recovery values obtained were closely similar to those published by Wolfe (19), but, as the result of further experience with the technique, were subject to smaller standard deviations (less than $\pm 2\%$ of the determined recoveries). After the separation of the amino acids, the further steps in their estimation were based on the method of Fowden (6), with the subsequent modifications made since that date and described by Wolfe (19). All determinations were made in triplicate.

Tryptophan was estimated directly on the protein samples by "procedure K" of Spies and Chambers (17), and proline was estimated after its separation on chromatograms by the method of Chinard (3).

Amide-nitrogen present in the protein was determined by a nesslerization procedure after its release as ammonia by treating the protein with *N* hydrochloric acid solution for 3 hours at 100°C.

Results and Discussion

The seven types of maize seed examined had similar total nitrogen and protein (calculated approximately as total nitrogen × 6.25) contents (see Table I). These values are typical of those determined for many other varieties of maize, and lie about the middle of the range (4.9–18.4% protein) that has been reported by Frey (9) for varieties selected for high or low protein content. Table I also records the total nitrogen and approximate protein content of each purified sample isolated from seed. It will be seen that purities of about 80% were obtained for all the protein fractions, assuming that the strictly pure protein would contain 16% by weight of nitrogen. Thus the fractionation procedure described above reduced the impurities, i.e. substances other than protein, from about 90% in the untreated ground seed to about 20% in the protein samples that were subjected later to acid hydrolysis.

It is rather surprising that the largest differences encountered between the total nitrogen content of the varieties examined occurred between the three types of K8 seed, since they were all from the same

TABLE I

TOTAL NITROGEN AND APPROXIMATE PROTEIN CONTENT OF ORIGINAL GROUND MAIZE SEEDS AND OF PURIFIED PROTEIN FRACTIONS ISOLATED FROM THESE SEEDS
(Total nitrogen content expressed in g. nitrogen per 100 g. air-dried sample)

VARIETY OF MAIZE	TOTAL NITROGEN CONTENT OF ORIGINAL SEED	APPROXIMATE PROTEIN CONTENT OF ORIGINAL SEEDS (TOTAL N × 6.25)	TOTAL NITROGEN CONTENT OF ISOLATED PROTEIN FRACTIONS	APPROXIMATE PROTEIN CONTENT OF ISOLATED PROTEIN FRACTIONS	ALPHA-AMINO NITROGEN AFTER 18-HOUR HYDROLYSIS AS PERCENT OF TOTAL PROTEIN NITROGEN
K8 red	1.65	10.3	12.50	78.1	74
K8 yellow	1.32	8.2	12.76	79.8	76
K8 white	1.53	9.6	12.73	79.6	75
Ulu flint	1.60	10.0	12.26	76.6	71
Ulu dent	1.59	9.9	12.80	80.0	73
Muratha white	1.46	9.1	13.34	83.4	72
Muratha yellow	1.54	9.6	12.73	79.6	71

cobs. These different nitrogen contents are unlikely to result from varying levels of nutrient supply during the maturing of the different colored grains, and presumably must be true biochemical differences of genetic origin.

When the protein fractions were hydrolyzed by the procedure described above, the proteins rapidly dissolved in the mixture of acids. No insoluble humin was formed during the hydrolyses, and the hydrolysates remained a light amber color at the end of the treatment. The yield of alpha-amino-nitrogen obtained from each protein sample after 18 hours' hydrolysis, expressed as a percentage of the total protein nitrogen present in the sample, is given in Table I. The percentages range from 71 to 76.

Protein samples giving lower yields of alpha-amino-nitrogen should contain relatively larger amounts of those amino acids which have two or more nitrogen atoms in their molecules, whereas those proteins which yield higher percentages of alpha-amino-nitrogen should contain lower amounts of these acids. Reference to the amino acid data in Table II confirms that this is generally true. The protein samples from Muratha yellow and Ulu flint maize give only 71% of the total protein-nitrogen as alpha-amino-nitrogen after hydrolysis; the protein of Ulu flint maize contains relatively large amounts of arginine and lysine, whereas that of Muratha yellow maize has high contents of arginine and amide-nitrogen. The protein of K8 yellow maize which yields 76% of its total nitrogen as alpha-amino-nitrogen on hydrolysis contains relatively small amounts of arginine, lysine, and amide-nitrogen. The fact that the amino acid composition is as expected in these respects provides a satisfactory check on their accuracy.

TABLE II

AMINO ACIDS PRESENT IN THE PROTEIN ISOLATED FROM VARIOUS MAIZE SEEDS

(Data expressed as g. of nitrogen in each amino acid per 100 g. total protein-nitrogen. Values are means of triplicate determinations ± the standard error of the mean[a])

AMINO ACID	K8 RED	K8 YELLOW	K8 WHITE	ULU FLINT	ULU DENT	MURATHA WHITE	MURATHA YELLOW
Alanine	6.0 ± 0.08	6.0 ± 0.04	5.3 ± 0.08	6.5 ± 0.06	7.3 ± 0.11	7.5 ± 0.10	7.5 ± 0.09
Arginine	8.7 ± 0.21	8.5 ± 0.20	8.5 ± 0.20	14.7 ± 0.20	14.7 ± 0.23	13.1 ± 0.21	13.7 ± 0.20
Aspartic acid	3.2 ± 0.04	3.8 ± 0.06	5.5 ± 0.03	4.0 ± 0.06	4.0 ± 0.07	3.7 ± 0.06	4.1 ± 0.06
Cyst(e)ine	2.3 ± 0.06	2.7 ± 0.07	2.7 ± 0.06	1.8 ± 0.05	2.1 ± 0.05	2.2 ± 0.04	3.0 ± 0.05
Glutamic acid	8.3 ± 0.14	8.2 ± 0.15	9.5 ± 0.07	9.4 ± 0.13	8.9 ± 0.14	7.9 ± 0.12	7.5 ± 0.13
Glycine	2.7 ± 0.03	3.3 ± 0.05	3.5 ± 0.03	4.6 ± 0.03	4.7 ± 0.04	3.9 ± 0.02	3.7 ± 0.03
Histidine	7.8 ± 0.18	6.6 ± 0.15	7.0 ± 0.15	5.9 ± 0.12	6.6 ± 0.14	4.8 ± 0.09	4.9 ± 0.10
Isoleucine	4.4 ± 0.04	4.9 ± 0.07	4.0 ± 0.06	3.1 ± 0.06	2.9 ± 0.05	4.4 ± 0.06	4.3 ± 0.06
Leucine	9.9 ± 0.08	9.5 ± 0.07	9.2 ± 0.10	7.7 ± 0.08	6.8 ± 0.09	11.0 ± 0.15	10.7 ± 0.12
Lysine	4.5 ± 0.11	4.2 ± 0.11	5.7 ± 0.12	7.1 ± 0.09	7.5 ± 0.13	4.3 ± 0.08	4.2 ± 0.09
Methionine	2.6 ± 0.05	2.8 ± 0.05	3.0 ± 0.05	3.2 ± 0.07	3.2 ± 0.07	3.3 ± 0.06	3.2 ± 0.07
Phenylalanine	5.6 ± 0.07	5.1 ± 0.09	4.3 ± 0.06	3.5 ± 0.08	3.5 ± 0.07	4.0 ± 0.08	3.9 ± 0.07
Proline	4.6 ± 0.06	3.3 ± 0.09	3.8 ± 0.08	5.0 ± 0.09	4.8 ± 0.09	4.8 ± 0.08	4.9 ± 0.07
Serine	3.2 ± 0.07	3.7 ± 0.08	4.1 ± 0.08	3.6 ± 0.08	3.7 ± 0.08	3.4 ± 0.07	3.6 ± 0.07
Threonine	6.6 ± 0.12	10.8 ± 0.05	9.7 ± 0.10	6.2 ± 0.09	6.5 ± 0.10	3.4 ± 0.06	2.1 ± 0.05
Tryptophan	0.77 ± 0.00	1.05 ± 0.015	0.74 ± 0.00	1.04 ± 0.009	0.77 ± 0.004	0.75 ± 0.006	0.72 ± 0.00
Tyrosine	2.2 ± 0.01	3.2 ± 0.08	3.2 ± 0.07	2.3 ± 0.06	2.2 ± 0.05	2.2 ± 0.06	2.0 ± 0.04
Valine	4.6 ± 0.08	6.2 ± 0.04	5.2 ± 0.08	3.0 ± 0.06	2.9 ± 0.07	4.0 ± 0.06	3.9 ± 0.04
Amide	8.0 ± 0.08	4.7 ± 0.04	4.7 ± 0.05	6.2 ± 0.05	5.6 ± 0.06	8.6 ± 0.08	8.5 ± 0.07
Total	96.0 ± 1.51	98.6 ± 1.51	99.6 ± 1.47	98.8 ± 1.47	98.7 ± 1.64	97.3 ± 1.49	96.4 ± 1.41

[a] These standard errors take account of the standard errors of the means for the determinations of the reference (standard) amino acids.

The full amino acid composition of each protein fraction is given in Table II together with standard errors of the means for each determination. Virtually all the protein-nitrogen has been accounted for in terms of the hydrolysis products. Direct comparison of these figures with earlier recorded values for amino acids in maize protein is difficult, owing to the fact that most previous investigations report the content of amino acids on the rather unsatisfactory basis (see Tristram, 18) of g. of amino acid per 100 g. protein and not on a nitrogen basis as here. Cystine, methionine, lysine, and tryptophan have been estimated most frequently (4, 8, 9, 12, 13, 14), and after adjustment of the reported values to the nitrogen basis used in Table II, the contents of the amino acids fall in the following ranges: cystine represents 1.0–1.6%, methionine 0.8–1.6%, lysine 2.9–4.1%, and tryptophan 0.58–1.02% of the protein-nitrogen. Our values for cystine, methionine, and lysine are invariably higher than the maximum values reported previously. Similarly, our values for histidine and tyrosine are higher than those reported in maize protein by Sauberlich *et al.* (14). It is highly significant that, while the earlier values for these five amino acids were obtained after hydrolysis of whole ground seed, it is precisely these acids that are partially destroyed when protein is hydrolyzed by acid in the presence of high concentrations of carbohydrate.

When the compositions of the protein of the maize varieties we have examined are compared, only small differences are apparent between the protein of different representatives of the three main genetic types. These differences are greatest in the three K8 varieties for aspartic acid, threonine, valine, and amide-nitrogen, and in Muratha strains for cyst(e)ine and threonine. No really striking differences of composition occur between the protein of Ulu flint and dent maize seed. Larger differences are apparent, however, when the composition of the protein of K8, Muratha, and Ulu maize seed is compared. Statistically the differences mentioned below were found to have less than 0.1% probability of occurring by chance.

The following general conclusions can be made:

1. The protein of Ulu and Muratha varieties contains considerably more arginine than the protein of the K8 varieties.
2. Ulu maize protein contains less leucine, isoleucine, and valine than the protein of either K8 or Muratha maize varieties, and is also characterized by relatively high contents of the three basic amino acids.
3. K8 and Ulu varieties have protein in which threonine and histidine are present in greater amounts than they are in the protein of Muratha maize seed.

These new analyses indicate that, relative to other noncereal seed and leaf protein, maize protein is deficient in tryptophan. The earlier reported deficiencies of lysine and valine no longer appear general for all maize varieties; Ulu maize seed has protein in which lysine is present in amounts equal to that of the protein of many leaves, while K8 seed has no marked deficiency of valine.

The differences between the proportions of certain amino acids in the protein of different maize seed, which are particularly striking for arginine, histidine, lysine, leucine, threonine, valine, and amide-nitrogen, cannot be ascribed merely to variations in the percentages of zein in the total seed protein for the following reasons. Zein contains relatively high amounts of glutamic acid, leucine, proline, and serine, and so marked changes in the percentages of this particular protein would be associated with simultaneous increases or decreases of the amounts of all these amino acids in the whole seed protein. The three basic amino acids, all relatively deficient in zein, should behave similarly, all being either relatively high or relatively low in the whole protein of any particular maize variety. Table II shows that no such correspondence between the levels of these amino acids occurs. Secondly the work of Mitchell *et al.* (12) shows that a change of 1% in the total protein content of maize seed is accompanied by a change of about 5% in the percentage of the total protein constituted by zein. Therefore differences in the zein contents of the maize varieties we have examined should be small (differences should not exceed 10% when expressed as percentage of the total protein), since the total protein contents of different varieties were closely similar (protein constituted 8.3–10.2% of the seed weights). Thus the conclusion to be drawn from the differing amino acid composition is that numerous other individual proteins, at present less precisely characterized than zein, occur in maize seed, and that these proteins are present in different varieties of maize in widely differing proportions. The individual proteins would be expected to include types having markedly different amino acid composition.

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A NEW MICRO MILL FOR PRELIMINARY MILLING-QUALITY TESTS OF WHEAT[1]

N. H. Shoup, K. L. Pell, E. F. Seeborg, and M. A. Barmore[2]

ABSTRACT

A two-break milling machine designed for the rapid milling-quality evaluation of 5-g. wheat samples is described. Essential components are two sets of break rolls, a feeding device, and a sifter. Four samples are milled simultaneously, and approximately 75 samples can be milled per hour.

A new "Five-gram milling-quality test" for use in screening large populations of early-generation breeding material will be described by Seeborg and Barmore in another paper.[3] The rapidly expanded use of this test at the Western Wheat Quality Laboratory made it desirable to design and construct a special mill for this purpose. The milling performance requirements are presented, with a discussion of the mill design developed to meet these specifications.

The essential mill components required for the 5-g. milling-quality test were: two sets of break rolls, a feeding device, and a sifter. Of importance in the arrangement and design of these components were the following requirements:

1. The completed milling operation must be performed without intermediate handling of stock.
2. Milling loss kept to a very low percentage.
3. Small 5-g. wheat samples fed into the break rolls rapidly and uniformly.
4. The mill must be self-cleaning between samples.
5. The capacity to process 500 or more samples a day.
6. Portable design.

Design Development

The mill components were mostly constructed from standard equipment. The feeder, break rolls, and sifter were arranged vertically to provide a continuous gravity flow through the mill. The roll faces were divided into five identical grinding sections in order to provide five multiple milling units. This was accomplished by cutting 1/8-in. grooves, 1/4 in. deep, in the rolls and inserting divider plates into

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[3] See article immediately following (*Cereal Chem. 34:* 299-303, 1957).

the grooves. The small roll sections minimized milling losses and the divider plates ensured that samples in adjacent sections did not intermix.

The bearings supporting the grinding rolls were fastened directly to the side frame without provision for the usual spring-loading of the rolls. This feature simplified the design considerably and contributed to the portability of the unit. This type of bearing mounting has had no apparent adverse effect on grinding performance.

Several alterations of original ideas were made after preliminary trials. The grooved feed roll did not work satisfactorily on such small (5-g.) samples, so it was replaced with a feeder consisting of a vibrating tray actuated by striker pins on the 1st break roll, as shown in Fig. 1. With proper vibration the trays convey a uniform flow of wheat from the feed hoppers to the break rolls.

Trials of several types of vibratory sifters designed for a stream-flow

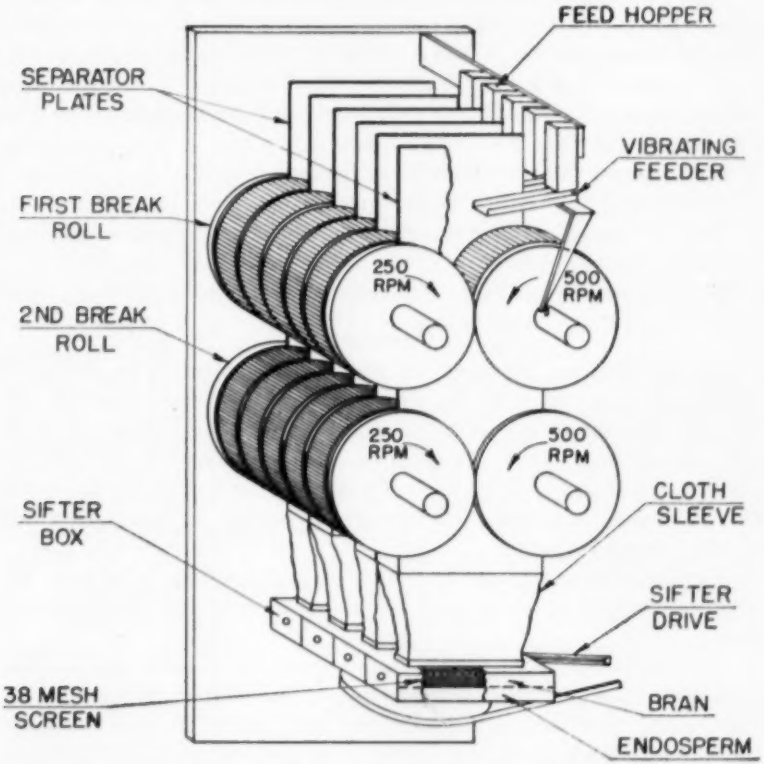

Fig. 1. Essential features of the mill with the case and bearings removed.

or inclined-screen system proved inadequate. This was due in part to space limitations in the mill as originally designed and also to the high moisture content of the ground meal. It was decided, at the expense of the convenience of stream-flow sifting, that a rotating eccentric box-screen system would be more satisfactory. With this method sifting time was accurately controlled with uniform treatment of the entire sample. It was found for this stock that a relatively slow rotary oscillation of the screen with a large throw was more satisfactory than a higher-frequency vibration of small amplitude.

The changes made in the sifting method limited the sifting operation to only four samples at a time. The mill capacity, however, was still about 75 samples per hour which was sufficient to meet the original requirements.

Mechanical Specifications

Figure 1 shows the main details of the mill schematically. The following specifications are listed for convenience.

Roll corrugations – 1st break 16 per in.; 2nd break 20 per in.; spiral 1 in. per ft.

Roll speed – 500 r.p.m. (fast roll) with 2-to-1 differential

Sifter speed – 215 r.p.m., ¾-in. throw

Sifter screen – No. 38 stainless-steel screen

Mill drive – 1-HP motor (sifter ¼-HP motor)

Roll tramming – Horizontal guide under bearing flanges with lock screw adjustment

Roll tension – Rigid bearing mounts, no spring

Operation

Four preweighed and tempered samples (described in the following article) are removed from the desiccator and placed in the hoppers, and the two motors are switched on. The roll motor is switched off after 30 seconds, but the sifter runs for an additional 30 seconds. Samples are removed from the sifter and the bran remaining on the No. 38 wire is weighed. The endosperm sample is either discarded or saved for other tests.

The entire operation can be completed in 3 minutes or less with one operator running the mill and another weighing and recording the results.

The mill described here has functioned satisfactorily while grinding a wide range of wheat milling types at the Western Wheat Quality Laboratory. Standard deviations have not exceeded those obtained by the batch method on the Buhler mill with micro attachment (see Seeborg, E. F., and Barmore, M. A. *Cereal Chem. 34:* 299–303, 1957).

A NEW FIVE-GRAM MILLING-QUALITY TEST AND ITS USE IN WHEAT BREEDING[1]

E. F. Seeborg and M. A. Barmore[2]

ABSTRACT

A milling-quality test employing 5 g. of wheat has shown evidence of usefulness in breeding programs in which an improvement in milling quality was one of the objectives. The test involves hydrating the samples to about 17.5% moisture, grinding them through two sets of break rolls, scalping the meal over a No. 38 wire to separate bran from endosperm, and weighing the bran. The result is an exaggerated difference in bran cleanup. Two operators can process 400 to 600 hydrated samples a day.

Previous work at several laboratories (2, 3, 5, 8) to reduce the sample size required for milling and baking determinations has partially succeeded. Data are obtained on smaller quantities of grain and several years earlier in the breeding programs than has previously been possible. A great number of samples in the fifth, sixth, and seventh generations are tested annually by the several wheat quality laboratories throughout the world by such methods. The size of the sample needed, usually from 100 to 2000 g., and the time required to mill each sample preclude maximum usefulness of such methods in breeding programs in which milling and baking quality are important objectives. Microscopic (4) and chemical (1) determinations available at the time of this study, while of great value, were also too time-consuming to be practical in screening large numbers of early-generation wheat-breeding lines.

Wide differences in milling characteristics exist among the varieties now being grown in the western part of the United States and among parental lines used in the breeding programs (6). The current use of parental varieties having superior milling quality in breeding new varieties in the Pacific Northwest emphasized the need for a rapid, small-scale milling-quality test.

The test described in this paper was developed to supplement the later larger-scale tests by preliminary evaluations of early-generation breeding material. Such early screening enables the wheat breeder to concentrate his performance tests on strains of suitable

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[2] Assistant milling technologist, A.E.S., Pullman, and Chemist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

milling types. Thus, new varieties that are obviously undesirable for milling would be eliminated from further consideration before reaching the "uniform nursery trial" stage of development.

Milling Procedure

The yield of the important milling fractions, flour, bran, and shorts, has been shown (7) to be a function of (a) the wheat moisture level during milling and (b) the variety. Flour remaining on the bran at increasing moisture levels, for example, increased more rapidly for the poorer milling varieties than for the better ones. Hence, at relatively high moisture levels differences in bran cleanup were larger, became easily apparent, and could be weighed and correlated with normal millings.

Preliminary trials using wheat tempered to 17.5% moisture had indicated that significant differences in bran weight and appearance between two varieties differing widely in this characteristic could be measured after passing a 5-g.[3] sample through the first two breaks of the Buhler mill, equipped with a micro feeder (8), and scalping the meal over a No. 38 wire screen. The scalping was done for 20 seconds on a 14 by 14-in. laboratory shaker subdivided to hold nine 4¼ by 4¼-in. sifter frames, running at 185 r.p.m. Removal of the flour and middlings between breaks or extending the grinding to three breaks did not improve the results.

Tempering the individual 5-g. samples from their normal moisture level near 9.0% to the desired level of 17.5 to 18.0% is accomplished by placing the samples in small envelopes without flaps in a cold-storage room maintained at 2–3° C. and near 100% relative humidity. By stacking the samples loosely into wire letter trays affording circulation, uniform hydration of large populations is obtained in from 10 to 12 days. Samples are at equilibrium at this 17.5 to 18.0% moisture level and have been held for as long as 6 months in such cold storage without any changes in milling properties occurring. Much more rapid hydration is possible at room or higher temperature, with the disadvantage, however, of favoring the growth of molds and other organisms in the wheat.

Sixteen hours prior to milling, a daily run of samples is transferred to desiccators charged with a saturated solution of zinc sulfate, thus maintaining the hydration level while bringing the samples to room temperature.

A newly designed two-break micro mill for use with this method is described by Shoup *et al.* (9).

[3] Five grams was selected as a weight of wheat which could normally be spared from a single plant while retaining sufficient seed for replanting.

Results

Twenty-five replicates of seven varieties milled in random order established the standard deviation in bran weight at 0.09 g., a coefficient of variation of about 4%. These replicate millings also showed that small differences in endosperm milling characteristics are not revealed. Difficulty in bran cleanup, however, is magnified.

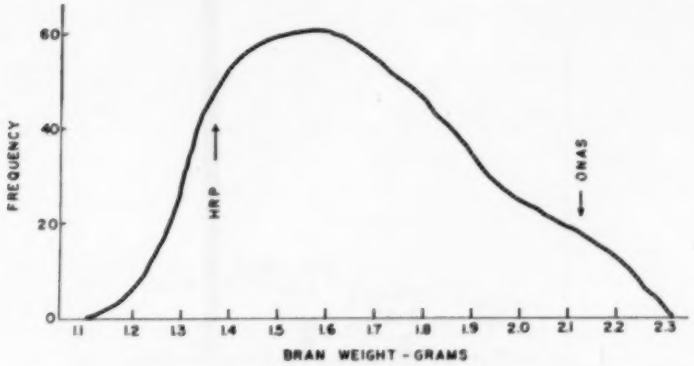

Fig. 1. Frequency curve for bran weights of 399 fourth-generation progeny of the HRP and Onas cross.

Figure 1 shows the frequency of bran weights from the milling of 399 progeny lines from a cross[4] that was designed to improve the milling characteristics of Awned Onas, in comparison with the mean weights of the parents. The data fall into a slightly skewed frequency curve. This indicates that bran cleanup is quantitatively inherited and may be governed by several genes. Some of the segregates were equal or superior to the better parent in this characteristic. Standard deviations were 0.04 and 0.09 g., respectively, for the checks HRP and Awned Onas.

Results from 1715 lines from the above cross that had been backcrossed to Awned Onas, the poor milling parent, are shown in Fig. 2. More than 88% of the backcross lines were similar in quality to the poorer parent, but 79 lines, or 4.6% of the total, were found to be significantly better than the recurrent parent. If a variety with the outstanding agronomic qualities of Awned Onas is obtained from one of these 79 lines, the value of the new testing method will have been established. The milling of these 1715 samples was accomplished by two operators in 4 days.

[4] The crosses and progeny used in this study were made and grown by the plant breeders of the Washington Agricultural Experiment Stations.

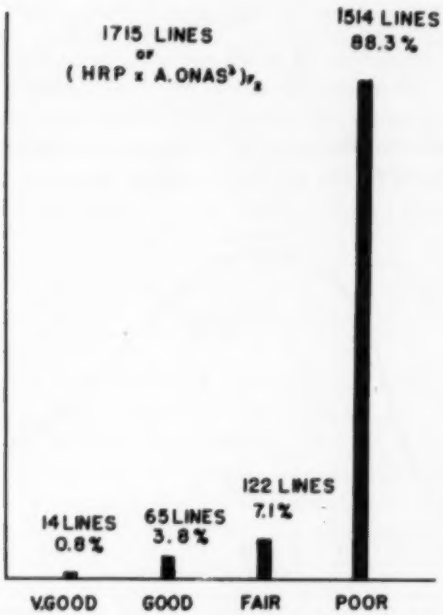

Fig. 2. Milling ratings based on bran weights of second-generation progeny of the HRP × Awned Onas × Awned Onas × Awned Onas cross.

A comprehensive study of the inheritance of bran cleanup is now under way jointly by the Washington Dry Land Experiment Station at Lind, Washington, and the Federal and State agronomists and the Western Wheat Quality Laboratory at Pullman, Washington. This will involve the milling of over 25,000 single plant lines in several generations following the original crosses, along with a number of backcrosses. Similar studies of a more limited scope are under way with widely divergent wheat types where the objective is to add a desirable agronomic character, e.g. short straw, from a poor milling parent, while retaining or improving on the milling quality of the other parent. A method that will evaluate 400 to 500 samples in a day makes such studies attractive to plant breeders and encourages them to devote more effort to breeding for better milling and baking quality.

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COMMUNICATION TO THE EDITOR

An Improved Method of Summarizing Flour Viscosity and Sedimentation Data

Dear Sir:

An improved method has been found for calculating the equation for the family of lines used to represent the changes in viscosity due to changes in protein level. A recent paper (*Cereal Chem.* **32:**144-152, 1955) suggests using the relation of the slope of the regression line to the constant of the regression equation for each variety. Instead, one should relate the slope to the viscosity at the standard protein level. (This laboratory uses 8% protein.) This method makes the family of lines fit the observed data better than the previous method.

Additional data have been obtained on the 17 varieties described in the above-mentioned study, and on 13 other varieties. With these, the correlation coefficient for slope of the protein-viscosity regression lines versus the viscosity at 8% protein was +0.714. The mean of the two regression equations with slope and viscosity as respective dependent variables was

$$C = 2.92\,b + 4.42 \qquad (2)$$

where C equals the viscosity at 8% protein and b equals the slope of the protein-viscosity regression equation for each of the 30 varieties.

Substitution of equation 2 for C in equation 1 in the original paper ($V = bP - C$, where V equals the viscosity and P equals the protein

content for any one variety) and rearranging gives

$$V - 4.42 = b\ (P - 5.08). \tag{3}$$

This is the equation of a family of lines intersecting at a viscosity of 4.42° and a protein content of 5.08% (the point at which b, the slope, can vary from 0 to ∞ without altering V or P).

Sedimentation data on the flour of 20 Western varieties by Zeleny's method (*Cereal Chem.* **24:** 465-475, 1947) revealed a similar fanning out of the regression lines from a common point. The correlation coefficient of the slope of the various regression lines and the sedimentation values at 11% protein content was +0.914. The mean regression equation was

$$C' = 5.91\ b' + 13.4 \tag{4}$$

where C′ equals sedimentation at 11% protein and b′ equals the slope of the sedimentation-protein regression line for each variety.

Substitution of equation 4 for C′ in a general equation for sedimentation and protein content ($S = b'P - c'$) and rearranging gives

$$S - 13.4 = b'\ (P - 5.1)$$

where S equals the sedimentation, b′ the slope of the regression line, and P the protein content of the flour.

Thus a means is available for correcting flour sedimentation data for differences in protein content similar to that developed for viscosity.

August 22, 1956

MARK A. BARMORE
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